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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Enhanced amylose production in plants

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Enhanced amylose production in plants

Description

The present invention relates to new amylose biosynthesis enhancing proteins, nucleic acids encoding a starch biosynthesis enhancing protein, a method for producing amylose with high efficiency by culturing genetically modified plants with an increased amylose biosynthesis compared to the wild type or to the genetically modified plants themselves as well as the use of these transgenic plants over-expressing at least one of the amylose biosynthesis enhancing proteins for the production of amylose.

Starch is the major storage carbohydrate of plants and is mainly accumulated in seeds and tubers, which are then the reproductive tissues of plants that form those types of organs. Starch is also accumulated on a diurnal basis where starch is built up in green tissue from photosynthetic products and then metabolised for energy during the dark period. The storage starch is assembled into semi crystalline granules. Amylopectin and amylose are the two constituent molecules of starch. Amylopectin is a branched molecule consisting of linear α-1,4 glucan chains linked by α-1,6 bonds. Amylose consists essentially of the linear α-1,4 glucan chains.

Starch is utilised for many applications within the technical industry as well as the food industry. Main crops used by starch processors are maize and potato. For potatoes specific varieties are utilised for starch production that have been bred for high starch contents. This means that the starch content and yield is an important economic driver for the starch processing industry. A greater part of produced dry starch is used for paper production. The specifications and requirements for the starch component varies from application to application and starch is many times chemically modified in order to provide desired properties to an application. Another way to achieve starch of different qualities is to take advantage of mutations in the starch biosynthesis and more recently by genetic modification of pathways leading to starch. The first main modifications have been to separate the production of the two starch components amylopectin and amylose into different varieties. Waxy or "amylose free" varieties contain solely amylopectin type starch while there are also high amylose genotypes such as "amylose extender" in maize.

Amylose starch has several potential industrial uses as a film former or for expanded products. High amylose starch can be achieved in potatoes and other starch containing plants by inhibition of starch branching enzymes. This leads then to the concomitant reduction or elimination of amylopectin branching and thereby an increased amylose fraction.

US 5,856,467 describes the genetically engineered modification of potato for suppressing formation of amylopectin-type starch. The document describes an antisense construct for inhibiting, to a varying extent, the expression of the gene coding for formation of starch branching enzyme (SBE gene) in potato, said antisense construct comprising a tuber specific promoter, transcription start and the first exon of the SBE gene, inserted in the antisense direction.

US 6,169,226 relates to an amino acid sequence of a second starch branching enzyme (SBE II) of potato and a fragment thereof as well as to the corresponding isolated DNA sequences. It describes the production of transgenic potatoes and the use of these transgenic potatoes for the production of amylose-type starch.

WO 97/20040 and WO 98/20145 describe methods of altering the amylopectine/ amylose starch content of plant cells by introducing into the plant cells nucleic acid sequences operably linked in sense or antisense orientation to a suitable promoter which homologous genes encodes polypeptides having SBE I or SBE II activity.

A side effect of the amylose overproduction is a decreased total starch content in the potatoes. This decrease becomes more pronounced as the amylose fraction is increased.

Basic enzymes for the production of amylopectin and amylose are starch synthases that build the linear α-1,4 glucan chains and branching enzymes breaking the α-1,4 glucan chain and reattaching them by α-1,6 bonds. Several other enzymes are likely to affect starch structure and composition, such as debranching enzymes, but initially most focus has been towards affecting the expression of starch synthases and starch branching enzymes. This has led to an extensive dissection of what enzymes are important for what features of starch synthesis. However it has never been convincingly shown how the synthesis of starch in plants whether amylose or amylopectin is initiated.

Suggestions on the initiation of starch biosynthesis have been the subject of several scientific papers since it has been difficult to attribute a primer independent function to starch synthases under other than artificial in vitro conditions. By primer independent function implies the formation of new α-1,4 glucan chains with ADP-glucose as the sole starting point and building block. One proposed pathway has been that the presence of maltooligosaccharides act as primers for the addition of further glucose units by starch synthases although it has been debated on whether concentrations are sufficient to provide the basis for starch synthesis and also how these maltooligosaccharides would be formed in the plastids.

Starch is in plants synthesised as an energy storage molecule. Much is known about the enzymes participating in the starch biosynthesis although, the initiation of the starch molecule. In mammalians and yeast an energy storage molecule very similar to starch is synthesised, glycogen. The enzymatic steps for synthesis of the respective molecules are analogous. In glycogen biosynthesis the initiation of the molecule is known and synthesised by the enzyme glycogenin. Glycogenin is a self-glucosylating enzyme polymerising a linear chain of approximately 8 glucose molecules on itself. The primer of about 8 glucose residues is necessary for the enzymes catalysing the continuation of glucose incorporation to the glycogen molecule to function.

Cheng et al., 1995, Mol. and Cell. Biol. 6632-6640 compare the two yeast proteins with rabbit muscle glycogenin.

Roach et al., 1997, Progress in Nucleic Acid Research and Molecular Biology Vol 57, describe self glycosylating initiator proteins and their roll in glycogen biosynthesis.

Mu et al., 1997, Journal of Biological Chemistry 272 (44), 27589-27597 compare mammalian with yeast and C. elegans glycogenins.

Factors important for starch quantity have been investigated and many initiatives have been taken, especially in potato, to increase starch formation and content by over-expression or inhibition of various enzyme activities in areas of increased substrate supply, increased biosynthesis activity or shutting down substrate diverting pathways but so far this has led only to limited success with no commercial applications and only some scientific publications.

Regierer, B. et al., Starch content and yield increase as a result of altering adenylate pools in transgenic plants. Nat Biotechnol. 20(12):1256-60, (2002).

Sweetlove, LJ et al., Starch synthesis in transgenic potato tubers with increased 3-phosphoglyceric acid content as a consequence of increased 6-phosphofructokinase activity. Planta 213(3):478-82 (2001).

Veramendi, J et al.., Antisense repression of hexokinase 1 leads to an overaccumulation of starch in leaves of transgenic potato plants but not to significant changes in tuber carbohydrate metabolism. Plant Physiol. 121(1):123-34 (1999).

Geigenberger, P et al.., Overexpression of pyrophosphatase leads to increased sucrose degradation and starch synthesis, increased activities of enzymes for sucrose-

starch interconversions, and increased levels of nucleotides in growing potato tubers. Planta. 205(3):428-37(1998).

Sweetlove, LJ et al., Starch metabolism in tubers of transgenic potato (Solanum tuberosum) with increased ADPglucose pyrophosphorylase. Biochem J. 320 (Pt 2):493-8 (1996).

In other research a biochemical function superficially similar to the one initiating glycogen production in animals was investigated. A class of genes have then been isolated from several plants and was given the name amylogenin (WO94/04693; Sing, D. et al; β-Glucosylarginin: a new glucose-protein bond in a self-glucosylating protein from sweet corn, FEBS Letters 376:61-64, (1995) in the belief that it was the plant equivalent of glycogenin which acts as a self-glycosylating enzyme and provide primers for starch biosynthesis in plants. These genes have no resemblance from a structural point of view to the genes coding for glycogenin and have later been determined not to have a function in starch biosynthesis but rather might be of importance for cell wall formation , see Bocca, S.N et al., Molecular cloning and characterization of the enzyme UDP-glucose: protein transglucosylase from potato. Plant Physiology and Biochemistry 37(11):809-819(1999).

WO 98/50553 describes nucleic acid fragments encoding a plant glycogenin or a water stress protein. WO 98/50553 also relates to the construction of chimeric genes encoding all or a portion of a plant glycogenin in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of a plant glycogenin in a transformed host cell.

Thus although many enzymes and pathways have been investigated in plants, the question on how starch formation is initiated and what determines the starch content is still unresolved.

Amylose is a commercially important starch product with many uses but unfortunately an increase in amylose content in transgenic potato plants is associated with a significant decrease in starch content, see figure 1.

Analyses of transgenic high amylose potato lines show that there is an excess of soluble sugars in these lines, see figure 2. This indicates that the starch biosynthesis in these transgenic lines is not efficient enough for incorporation of available sugars.

Amylose starch consists of very few reducing ends compared to native starch. Therefore it is commercially important to identify genes that further enhance the amylose

biosynthesis and that are capable to incorporate the excess of glucose residues available and to compensate the decrease in starch content in plants that produces amylose in high amounts.

The invention aims at enhancing the yield of amylose biosynthesis by the over-expression of genes which enhance amylose biosynthesis in transformed plants.

The invention describes genes coding for proteins which enhance amylose production.

The present invention describes the nucleic acids SEQ ID NO 1 and 3 from potato coding for enzymes enhancing the de novo amylose biosynthesis.

Example 1 describes that the nucleic acid sequences SEQ ID NO 1 or 3 can complement a missing glycogenin function in yeast cells containing knock-out mutations for the self-glycosylating proteins Glg1p and Glg2p.

Gene constructs were made for gene-inhibition and over-expression of the two genes SEQ ID NO 1 or 3 in potato. Transgenic lines with the over-expressed or inhibited enzyme activity were analysed with regard to the genes influence on amylose content.

Both genes were inserted in sense and antisense direction downstream of a plant promoter element, resulting in the transformation binary vectors pHS1, pHS2, pHS3 and pHS4, see figures 3-7.

The antisense constructs were transformed into potato plant varieties Prevalent and Producent and the sense constructs were transformed to the potato varieties Desiree and the transgenic plant AM99-2003 according to the transformation method as described in example 2. The transgenic plant AM99-2003 was produced as described in example 3.

Prevalent and Producent are starch varieties having a starch content of approximately 20 %. Desirée is a potato variety having a starch content of approximately 16% and AM99-2003 is a transgenic high amylose line having a starch and thereby amylose content of approximately 13%.

The putative genes were isolated from a tuber specific cDNA library of Solanum tuberosum (variety Prevalent). The library was made from a lambdaZAP directional kit (Stratagene).

Both cDNAs isolated were full-length clones of the individual genes and named StGH1 and StGH2, for nucleic acid sequences see SEQ ID NO 1 and SEQ ID NO 3.

pHS1

A 1300bp PCR fragment from the StGH1 gene was constructed in antisense direction driven by the gbss promoter. The PCR fragment was cut out from its cloning vector pCR4-TOPO (Invitrogen) with EcoRI (blunted) and XbaI. The fragment was ligated to the pGPTV-kan (Becker, D. et al., Plant Molecular Biology 20:1195-1197(1992) based binary vector pHo3.1 between a gbss promoter (WO 92/11376) and a nos terminator at the SalI (blunted) and XbaI sites. The binary vector also includes nptII as selection marker driven by the nos promoter (Herrera, L. et al., 1983). The construct was named pHS1, for details see figure 3a and 4.

pHS2

A 2300bp full-length cDNA clone of StGH2 was cut out from the cloning vector pBluescript (Stratagene) with XbaI and XhoI. The gene was ligated in antisense direction between the gbss promoter and nos terminator to the binary vector pHo3.1 at XbaI and SalI. As can be seen under pHS1 the vector has nptII as selection system. The vector was named pHS2, for details see figure 3b and 5.

pHS3

A full-length StGH1 cDNA, (1780bp) was cut out from the host vector pBluescript with EcoRI (blunted) and BglII and ligated to the BamHI and SmaI sites of pUCgbssprom (3886bp), containing pUC19 with the gbss promoter and the nos terminator. The plasmid was named pUCGH1.

A fragment with the gbss promoter, the StGH1 gene and the nos terminator was moved from pUCGH1 with EcoRI (blunted) and HindIII (2980bp) and ligated to PstI (blunted) and HindIII opened pSUN1 (WO 02/00900). The plasmid was named pSUNGH1.

A 3600bp fragment containing the AHAS resistance gene from Arabidopsis thaliana (Sathasivan, K. et al., Plant Physiology 97(1991), 1044-1050) with nos promoter, see Herrera-Estrella, L. et al., Nature 303:209-213(1983) and OCS terminator (Wesley, S.V. et al., Plant J. 27(6):581-590(2001) was ligated to pSUNGH1 (9000bp) at the SmaI site. The vector was given the name pHS3, for details see figure 3c and 6.

pHS4.

The gbss promoter and nos terminator was ligated to pBR322 with EcoRI and HindIII. Between the promoter and terminator an EcoRI-HincII full-length gene pStGH2 was cloned at the XbaI site. The 3366bp promotor-gene-terminator complex was cut using EcoRI (partial digestion) and EcoRV, and ligated to pSUN1 at EcoRI-EcoRV and named pSUNGH2. An XbaI fragment with AHAS gene (Arabidopsis thaliana), nos promoter and OCS terminator was ligated to pSUNGH2 opened with XbaI (partial digestion). The AHAS gene is used as selection marker. The construct was named pHS4, for details see figure 3d and 7.

Example 2 describes the general method for the transformation of different potato plant varieties producing native starch or high amylose type starch with pHS1, pHS2, pHS3 or pHS4.

The StGH1 and StGH2 genes were down-regulated in the potato plant varieties Prevalent and Producent by transformation with the genes in antisense direction in relation to a plant regulatory element as described in example 4 and 6. Down-regulation of the two genes resulted in a decrease in gene expression in transgenic lines, compared to their mother varieties in the order of 50-95%, see example 7 and table 3. Transgenic lines transformed with pHS1 and pHS2 with confirmed decrease in gene-expression have a decrease in dry matter of 7 to 11% compared to their mother varieties, see example 8 and table 5.

The StGH1 and StGH2 genes were over-expressed in potato driven by the tuber specific promoter gbss, as described in example 5. A mutated AHAS gene was used as selection marker yielding tolerance to the Imazamox herbicides. Two potato varieties were transformed, Desiree and AM99-2003 a transgenic high amylose line with a 40% decrease in starch content compared to its mother variety. The transformed lines over-expressing StGH1 and StGH2 were selected as described in example 6. The gene expression levels were analysed with real-time PCR, see example 7 and table 3. The over-expression of the genes StGH1 and StGH2 resulted in an 2 two10 times increase in gene compared to their mother variety. Furthermore the lines over-expressing StGH1 and StGH2 showed an increase in dry matter of up to 36 % as described in example 8 and table 5.

The over-expression of StGH1 and StGH2 in transgenic potato plants producing amylose type starch resulted in an increased dry matter content, which means an increased amylose content as no amylopectine is produced, see example 8, 9 and 11.

The amylose biosynthesis enhancing protein according to the invention comprises the amino acid sequence SEQ ID NO 2 or 4 or a protein which comprises a sequence derived from SEQ ID NO 2 or 4, which is at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level to the sequence SEQ ID NO 2 or 4 and has the property of an amylose biosynthesis enhancing protein. This amylose biosynthesis enhancing protein may also be prepared by artificial variations starting from the SEQ ID NO 2 or 4, for example by substitution, insertion or deletion of amino acids.

Such a protein could also be used to increase the production of starch or amylopectin in non-transgenic or transgenic plants.

The term "substitution" in the specification means the replacement of one or more amino acids by one or more amino acids. Preference is given to carrying out "conservative" replacements in which the amino acids replaced has a property similar to that of the original amino acid, for example replacement of Glu by Asp, Gln by Asn, Val by Ile, Leu by Ile, Ser by Thr.

"Deletion" is the replacement of an amino acid or amino acids by a direct bond. Preferred positions for deletions are the polypeptide termini and the junctions between the individual protein domains.

"Insertions" are insertions of amino acids into the polypeptide chain, with a direct bond formally being replaced by one or more amino acids.

"Identity" between two proteins means the identity of the amino acids over the in each case entire length of the protein, in particular the identity which is calculated by comparison with the aid of the Vector NTI Suite 7.1 Software of the company Informax (USA) using the Clustal W method (Thompson, JD et al., Nucleic Acid Research, 22 (22):4673-4680, 1994)

with the parameters set as follows:

Multiple alignment parameter:

Gap opening penalty	15
Gap extension penalty	6.66
Gap separation penalty range	8
Gap separation penalty	on

% identity for alignment delay	40
Residue specific gaps	on
Hydrophilic residue gap	off
Transition weighing	0

Pairwise alignment parameter:

FAST algorithm	off
K-tuple size	2
Gap penalty	5
Window size	4
Number of best diagonals	4

Accordingly, a protein which is at least 50% identical at the amino acid level to the sequence SEQ ID NO 2 or 4 means a protein which, when comparing its sequence with the sequence SEQ ID NO 2 or 4, is at least 50% identical, in particular according to the above program algorithm using the above set of parameters.

Further natural examples of genes coding for an amylose enhancing protein according to the invention can readily be found, for example, in various organisms, in particular in plants, whose genomic sequence is known by comparing the identity of the amino acid sequences or of the corresponding back-translated nucleic acid sequences from databases with the sequence of SEQ ID NO 2 or 4, in particular according to the above program algorithm using the above set of parameters.

In the completed genome sequence of Arabidopsis thaliana, five putitative coding sequences can be deduced by searching for exon/intron boundaries and comparing with back translated sequences of SEQ ID NO 2 or 4.

The following nucleic acid sequences of Arabidopsis thaliana SEQ ID NO 5, SEQ ID NO 7, SEQ ID NO 9, SEQ ID NO 11 and SEQ ID NO 13 could be used to carry out the invention and are coding for the amylose biosynthesis enhancing proteins SEQ ID NO 6, SEQ ID NO 8, SEQ ID NO 10, SEQ ID NO 12 and SEQ ID NO 14.

Furthermore the following nucleic acid sequences or ESTs can be used in order to identify and clone genes coding for an amylose biosynthesis enhancing protein from plant organisms:

Tomato ESTs from GenBank: AW216407, BE450055, BF097262, BE450557, BF097173

Wheat ESTs from GenBank: BJ292476, BJ278875, BJ283925, BE442966, CA666180, BQ483228

Maize EST from GenBank: BG319971

Rice ESTs from GenBank: AL606633, CA752890, BI813265

Natural examples of amylose biosynthesis enhancing proteins and the corresponding genes can furthermore readily be found in various organisms, in particular plants, whose genomic sequence is unknown by hybridization techniques in a manner known per se, for example starting from the nucleic acid sequences SEQ ID NO 1 or SEQ ID NO 3 or any of the SEQ ID NO 5, 7, 9, 11 or 13 or any of the EST sequences described above.

The hybridization may be carried out under moderate (low stringency) or, preferably, under stringent (high stringency) conditions.

Such hybridization conditions are described, inter alia, in Sambrook, J., Fritsch, E.F., Maniatis, T., in: Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57 or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

By way of example, the conditions during the washing step may be selected from the range of conditions which is limited by those with low stringency (with 2X SSC at 50°C) and those with high stringency (with 0.2X SSC at 50°C, preferably at 65°C) (20X SSC: 0.3 M sodium citrate, 3 M sodium chloride, pH 7.0).

In addition, the temperature may be raised during the washing step from moderate conditions at room temperature, 22°C, to stringent conditions at 65°C.

Both parameters, salt concentration and temperature, may be varied simultaneously and it is also possible to keep one of the two parameters constant and to vary only the other one. It is also possible to use denaturing agents such as, for example, formamide or SDS during hybridization. In the presence of 50% formamide, the hybridization is preferably carried out at 42°C.

Some exemplary conditions for hybridization and washing step are listed below:

(1) hybridization conditions with, for example

(i) 4X SSC at 65°C, or

(ii) 6X SSC at 45°C, or

(iii) 6X SSC at 68°C, 100 mg/ml denatured fish sperm DNA, or

(iv) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA at 68°C, or

(v) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA, 50% formamide at 42°C, or

(vi) 50% formamide, 4X SSC at 42°C, or

(vii) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrate at 42°C, or

(viii) 2X or 4X SSC at 50°C (moderate conditions), or

(ix) 30 to 40% formamide, 2X or 4X SSC at 42°C (moderate conditions).

(2) Washing steps of 10 minutes each with, for example

(i) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C, or

(ii) 0.1X SSC at 65°C, or

(iii) 0.1X SSC, 0.5% SDS at 68°C, or

(iv) 0.1X SSC, 0.5% SDS, 50% formamide at 42°C, or

(v) 0.2X SSC, 0.1% SDS at 42°C, or

(vi) 2X SSC at 65°C (moderate conditions).

Preferred proteins with amylose biosynthesis enhancing activity are proteins from plants, cyanobacteria, mosses or algae, particular preferred from plants. A particular preferred protein comprises the amino acid sequence SEQ ID NO 2 or 4.

If, for example, the protein is to be expressed in a plant, it is frequently advantageous to use the codon usage of said plant for backtranslation and resynthesis of the gene according to codon usage of said plant.

The invention further relates to nucleic acids encoding an amylose biosynthesis enhancing protein according to the invention. All of the nucleic acids mentioned in the specification may be, for example, a RNA sequence, DNA sequence or cDNA sequence.

Suitable nucleic acid sequences can be obtained, for example, by back-translating the polypeptide sequence according to the genetic code. For this, preference is given to using those codons which are used frequently according to the organism-specific codon usage. The codon usage can be readily determined on the basis of computer analyses of other known genes of the organisms in question.

All of the above-mentioned genes coding for an amylose biosynthesis enhancing protein can furthermore be prepared in a manner known per se from the nucleotide building blocks by chemical synthesis, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). The annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

Genes coding for this function may be integrated in the plant chromosomes and upon expression utilize a transit peptide to localise to plastids which is the organelle where amylose biosynthesis takes place or be integrated directly into the plastid genome and thereby surpass the need for the localisation signal. The genes may be expressed constitutively or organ specific. For organ specific expression, promoters with tuber specific expression is preferable in potatoes while in cereals as maize or wheat a endosperm specific expression would be preferred to achieve a high degree of expression in organs where storage starch is accumulated. When transformed to the plastid genome then specific regulatory elements suitable for that organelle apply.

The genes of this invention may be used in combination with other genes that can be situated on the same gene construct or transferred and combined by co-transformation or super transformation. Genes and traits that are of interest to combine with the genes of the instant invention are agronomic or input trait such as herbicide tolerance, disease and pest resistance or stress tolerance but could also be output traits such as starch structure modification or yield. Genes and traits used in combination with the genes described in the invention could be for adding a function that is not present in the modified plant species or over-expressing a function that is already present or inhibiting a function by the use of antisense, RNAi or antibodies.

The invention may be used to increase the amylose content in potato tubers but would in its context not be limited to potatoes but would be applicable to other starch producing and storing plants such as e.g. corn, cassava, wheat, barley, oat and rice.

The described invention is particularly suited for eliminating a lower starch content associated with increased amylose content in different plants where the number of α-1,4-glucan chain non-reducing ends is greatly reduced due to the reduction or elimination of α-1,6 branch formation. Amylopectin is an extremely efficient structure, as is glycogen, for polysaccharide production since it is very branched and thus contains as many points accessible for starch synthesis as there are non-reducing ends. Starch that is mainly composed of amylose, contains much fewer branches and thus the biosynthetic capacity is reduced. In order to enhance starch biosynthesis when there is no amylopectin production, expression of genes as described in the present invention, could for example form new primers that can replace amylopectin as a source for starch biosynthesis capacity and thereby reduce or eliminate the lost capacity for starch synthesis. To further illustrate the situation the degree of branching in ordinary potato starch is approximately 3.1% while in high amylose starch it is 0.3-1.0% depending on amylose content this decrease of branching and starch content is further associated with an increase in glucose and fructose content.

The increased amylose content and thereby solids content is also advantageous for the processing properties in various applications such as for french fries, potato crisps and other potato based products. In addition to an increased solid content, the inserted genes SEQ ID NO 1 or 3 of the present invention result in the transformation of excess sugars into α-1,4-glucan chains and thereby reducing browning of fried potato products, Maillard reaction, in which amino acids react with free sugars.

Furthermore

(i) any gene of plant origin with the described activity can be used for increasing amylose content and solids

(ii) the genes can be controlled by any regulating promoter element functional in plant.

(iii) any starch producing crop of any variety can be transformed with the described genes.

(iiii) any plant transformation method can be used.

(iiiii) any binary vector can be used for the insertion of the described genes.

(iiiiii) the described genes can be combined with any other desired transgenically inserted traits.

The invention further relates to a method for producing amylose by culturing plants which have, compared to a wild type or a genetically modified plant producing already amylose type starch, an increased amylose biosynthesis activity, said proteins comprising the amino acid sequence SEQ ID NO 2 or 4 or a sequence which is derived from one of these sequences by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ ID NO 2 or 4.

Increased amylose biosynthesis activity compared to the wild type or transgenic line means that the amount of amylose formed is increased by the amylose biosynthesis enhancing protein in comparison with the wild type or transgenic line.

This increase in amylose biosynthesis activity is preferably at least 5%, further preferably at least 10%, further preferably at least 20%, further preferably at least 50%, more preferably at least 100%, still more preferably at least 200%, in particular at least 500%, of the protein activity of the wild type or transgenic line.

A "wild type" means the corresponding genetically unmodified starting plant. This plant is preferably Solanum tuberosum.

Depending on the context, the term "plant" means a wild type starting plant or a genetically modified starting plant.

"Transgenic plant" or "genetically modified plant" means that the plant contains an additional inserted gene segment that may be foreign or endogenous to the plant species, additional genes or additional gene fragments in sense and/or antisense orientation to a suitable promoter corresponding to the following polypeptides and showing enzymatic activity of a starch branching enzyme I, a starch branching enzyme II and/or the amylose biosynthesis enhancing protein as specified in SEQ ID NO 1 or 3 or polynucleotides having at least 60 % sequence identity thereof.

"Amylose type starch" means that the amylose content of the starch is increased compared to the amylose content of starch produced by wild type plants especially wild type potato plants.

The amylose biosynthesis activity may be increased in various ways, for example by eliminating inhibiting regulatory mechanisms at the translation and protein levels or by increasing the gene expression of a nucleic acid encoding a amylose biosynthesis enhancing protein compared to the wild type or transgenic plant, for example by inducing a gene encoding the amylose biosynthesis enhancing protein via activators or by introducing into the plant nucleic acids encoding a amylose biosynthesis enhancing protein.

According to the invention, increasing the gene expression of a nucleic acid encoding a amylose biosynthesis enhancing protein could also mean manipulating the expression of the endogenous amylose biosynthesis enhancing protein intrinsic to the plant, in particular in potato plants. This may be achieved, for example, by modifying the promoter DNA sequence of genes encoding an amylose biosynthesis enhancing protein. Such a modification which leads to a modified or preferably increased rate of expression of at least one endogenous gene encoding an amylose biosynthesis enhancing protein may be carried out by deleting or inserting DNA sequences.

It is also possible to modify expression of one or more endogenous amylose biosynthesis enhancing protein by applying exogenous stimuli. This may be carried out by particular physiological conditions, i.e. by applying foreign substances.

Furthermore, it is possible to achieve a modified or increased expression of at least one endogenous gene encoding an amylose biosynthesis enhancing protein by the interaction of a regulatory protein which is modified or is not present in the untransformed plant.

In a preferred embodiment, the amylose biosynthesis enhancing protein activity is increased compared to the wild type or transgenic plant by increasing the gene expression of a nucleic acid encoding an amylose biosynthesis enhancing protein, said amylose biosynthesis enhancing protein comprising the amino acid sequence SEQ ID NO 2 or 4 or a sequence which is derived from said sequences by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ ID NO 2 or 4.

In the case of genomic nucleic acid sequences encoding an amylose biosynthesis enhancing protein from eukaryotic sources, which contain introns, preferably already

processed nucleic acid sequences such as the corresponding cDNAs are to be used, if the host organism is unable to or cannot be enabled to express the corresponding amylose biosynthesis enhancing protein.

In this preferred embodiment, the transgenic plant of the invention thus contains, compared to the wild type or transgenic plant, at least one further gene encoding an amylose biosynthesis enhancing protein. In this preferred embodiment, the genetically modified plant of the invention has accordingly at least one transgenic endogenous or exogenous nucleic acid encoding an amylose biosynthesis enhancing.

Suitable and preferred nucleic acids are described above. In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ ID NO 1 or 3 is introduced into the plant.

According to the invention, organisms means preferably eukaryotic organisms, such as, for example, yeasts, algae, mosses, fungi or plants, which are capable of producing starch or amylose, either as wild type or enabled by genetic modification. Preferred organisms are photosynthetically active organisms such as, for example, plants which, even as a wild type, are capable of producing starch or amylose type starch.

Particularly preferred organisms are potato plants.

The present invention furthermore relates to the use of proteins comprising the amino acid sequence SEQ ID NO 2 or 4 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ ID NO 2 or 4 and having amylose biosynthesis enhancing activity.

The present invention further relates to the use of nucleic acids SEQ ID NO 1 or 3 or one of the SEQ ID NOs 5, 7, 9, 11 or 13 encoding proteins having an amylose biosynthesis enhancing activity in plants.

The transgenic organisms, in particular plants, are preferably prepared by transforming the starting organisms, in particular plants, with a nucleic acid construct containing the above-described nucleic acid, encoding an amylose biosynthesis enhancing protein which is functionally linked to one or more regulatory signals ensuring transcription and translation in said organisms.

Empfangszeit 7.Mär 16:43

These nucleic acid constructs in which the coding nucleic acid sequence is functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, in particular in plants, are also referred to as expression cassettes herein below.

Accordingly, the invention further relates to nucleic acid constructs, in particular to nucleic acid constructs functioning as expression cassette, which comprise a nucleic acid encoding an amylose biosynthesis enhancing protein which is functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, in particular in plants.

The regulatory signals preferably comprise one or more promoters ensuring transcription and translation in organisms, in particular in plants.

The expression cassettes include regulatory signals, i.e. regulatory nucleic acid sequences, which control expression of the coding sequence in the host cell. According to a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for at least one of the above-described genes located in between. Operative linkage means the sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence.

When the organism used is a plant, the nucleic acid constructs and expression cassettes of the invention preferably contain a nucleic acid encoding a plastid transit peptide ensuring localisation in plastids.

The preferred nucleic acid constructs, expression cassettes and vectors for plants and methods for preparing transgenic plants and also the transgenic plants themselves are described in examples 2 to 6 below.

The sequences preferred for operative linkage, but not limited thereto, are targeting sequences for ensuring subcellular localisation to plastids such as amyloplasts or chloroplasts but could also mean in the apoplasts, in the vacuole, in the mitochondrion, in the endoplasmic reticulum (ER), in the nucleus, in elaioplasts or in other compartments and translation enhancers such as the tobacco mosaic virus 5'-leader sequence (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

A suitable promoter of the expression cassette is in principle any promoter which is able to control the expression of foreign genes in plants.

"Constitutive" promoter means those promoters which ensure expression in numerous, preferably all, tissues over a relatively long period of plant development, preferably during the entire plant development.

Preference is given to using, in particular, a promoter from plants or a promoter originating from a plant virus. Preference is in particular given to the promoter of the 35S transcript of the CaMV cauliflower mosaic virus (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221-228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202).

Another suitable constitutive promoter is the Rubisco small subunit (SSU) promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No. X03677), the Agrobacterium nopaline synthase promoter, the TR double promoter, the agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich wheat protein (WO 91/13991), the Pnit promoter (Y07648.L, Hillebrand et al. (1998), Plant. Mol. Biol. 36, 89-99, Hillebrand et al. (1996), Gene, 170, 197-200) and other promoters of genes whose constitutive expression in plants is known to the skilled worker.

The expression cassettes may also contain a chemically inducible promoter (review: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108) which may be used to control expression of the amylose biosynthesis enhancing protein gene in the plants at a particular time. Promoters of this kind, such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), salicylic acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracycline-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic acid-inducible promoter (EP 0 335 528) and an ethanol- or cyclohexanone-inducible promoter (WO 93/21334), may likewise be used.

Further examples of suitable promoters are fruit ripening-specific promoters such as, for example, the fruit ripening-specific promoter from tomato (WO 94/21794, EP 409 625). Development-dependent promoters partly include the tissue-specific promoters, since individual tissues are naturally formed in a development-dependent manner.

Furthermore, preference is given in particular to those promoters which ensure expression in tissues or parts of the plant, in which, for example, biosynthesis of starch or amylose or of the precursors thereof takes place. Preference is given, for example, to promoters with specificities for leaves, stems, roots, seeds and tubers.

Seed-specific promoters are, for example, the phaseoline promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), the promoter of the 2S albumin gene (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), the legumin promoter (Shirsat A et al. (1989) Mol Gen Genet 215(2): 326-331), the USP (unknown seed protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), the promoter of the napin gene (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the sucrose-binding protein promoter (WO 00/26388) and the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Baeumlein et al. (1992) Plant Journal 2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980) and the vicillin promoter (Weschke et al. 1988, Biochem. Physiol. Pflanzen 183, 233-242; Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67).

Further suitable seed-specific promoters are those of the genes coding for high molecular weight glutenine (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase) and starch synthase. Preference is further given to promoters which allow seed-specific expression in monocotyledons such as e.g. corn, barley, wheat, rye, rice, etc. It is also possible to use advantageously the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promotors described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the glutelin gene, the zein gene, the kasirin gene and the secalin gene).

Examples of tuber-, storage root- or root-specific promoters are the patatin promoter class I (B33), the potato cathepsin D inhibitor promoter and the potato granular bound starch synthase (GBSS) promoter as described in EP-A 0 921 191.

Examples of leaf-specific promoters are the cytosolic FBPase promoter from potato (WO 97/05900), the rubisco (ribulose-1,5-bisphosphate carboxylate) SSU (small subunit) promoter and the potato ST-LSI promoter (Stockhaus et al. (1989) EMBO J 8:2445-2451).

Further promoters suitable for expression in plants have been described (Rogers et al. (1987) Meth in Enzymol 153:253-277; Schardl et al. (1987) Gene 61:1-11; Berger et al. (1989) Proc Natl Acad Sci USA 86:8402-8406).

The site of starch and amylose biosynthesis in potato plants is the amyloplast. Therefore amyloplast-specific targeting and activity of the gene products of the inventivenucleic acids SEQ ID NO 1 or 3 encoding an amylose biosynthesis enhancing protein is desirable.

The expression may also take place in a tissue-specific manner in all parts of the plant.

A further preferred embodiment therefore relates to a tuber-specific expression of the nucleic acids SEQ ID NO 1 or 3.

In addition, a constitutive expression of the gene encoding an amylose biosynthesis enhancing protein is advantageous. On the other hand, however, an inducible expression of this gene may also be desirable.

An expression cassette is preferably prepared by fusing a suitable promoter to an above-described nucleic acid encoding an amylose biosynthesis enhancing protein and, preferably, to a nucleic acid which has been inserted between promoter and nucleic acid sequence and which codes for an amyloplast-specific transit peptide and also to a polyadenylation signal according to familiar recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

Particular preference is given to inserted nucleic acid sequences which ensure targeting in the amyloplasts.

It is also possible to use an expression cassette in which the nucleic acid sequence encodes an amylose biosynthesis enhancing protein fusion protein, one part of the fusion protein being a transit peptide which controls translocation of the polypeptide. Preference is given to amyloplast-specific transit peptides which, after translocation of amylose biosynthesis enhancing protein into the amyloplasts, are enzymatically cleaved off the amylose biosynthesis enhancing protein part.

Particular preference is given to the transit peptide which is derived from the Nicotiana tabacum plastid transketolase or from another transit peptide (e.g. the transit peptide of the rubisco small subunit or of ferredoxin NADP oxidoreductase and also of isopentenyl pyrophosphate isomerase-2) or from its functional equivalent.

Further examples of a plastid transit peptide are the transit peptide of the plastid isopentenyl pyrophosphate isomerase-2 (IPP-2) from Arabidopsis thaliana and the transit peptide of the ribulose bisphosphate carboxylase small subunit (rbcS) from pea (Guerineau, F, Woolston, S, Brooks, L, Mullineaux, P (1988) An expression cassettte for targeting foreign proteins into the chloroplasts. Nucl. Acids Res. 16: 11380).

Plant genes of the invention which encode a plant amylose biosynthesis enhancing protein may already contain the nucleic acid sequence which encodes a plastid transit peptide. In this case, a further transit peptide is not required. For example, the Solanum tuberosum sequences of the amylose biosynthesis enhancing protein of the invention SEQ ID NO 1 or 3 contain already a transit peptide sequence.

The nucleic acids of the invention may be prepared synthetically or obtained naturally or comprise a mixture of synthetic and natural nucleic acid components and may also be composed of various heterologous gene sections of various organisms.

As described above, preference is given to synthetic nucleotide sequences with codons which are preferred by plants. These codons which are preferred by plants may be determined from codons which have the highest frequency in proteins and which are expressed in most of the interesting plant species.

When preparing an expression cassette, it is possible to manipulate various DNA fragments in order to obtain a nucleotide sequence which expediently can be read in the correct direction and is provided with a correct reading frame. The DNA fragments may be linked to one another by attaching adaptors or linkers to said fragments.

It is furthermore possible to use manipulations which provide appropriate restriction cleavage sites or which remove excess DNA or restriction cleavage sites. In those cases for which insertions, deletions or substitutions such as, for example, transitions and transversions are suitable, in vitro mutagenesis, primer repair, restriction or ligation can be used.

Preferred polyadenylation signals are polyadenylation signals functional in plants, exemplified by those which correspond essentially to T-DNA polyadenylation signals from Agrobacterium tumefaciens, in particular of the T-DNA gene 3 (octopine synthase).

or OCS terminator, the complete sequence of the Ti plasmid pTiACH5 (Gielen et al., EMBO J. 3, 835 –846(1984) or functional equivalents.

The invention further relates to the use of the nucleic acids SEQ ID NO 1 or 3 for increasing the starch or amylose content in plants, e.g. potato plants which, as wild type, are capable of producing starch or amylose, see examples 2-12.

The invention is not limited to the over-expression of the nucleic acid sequences SEQ ID NO 1 or SEQ ID NO 3 in plants especially potato plants.

The over-expression of both nucleic acid sequences SEQ ID NO 1 and 3 can be used for enhancing amylose biosynthesis. Constructs containing the nucleic acids SEQ ID NO 1 and SEQ ID NO 3 can also be used for increasing the starch content or the amylopectin content in plants. These constructs can be made on the same T-DNA driven by one promoter each. These constructs can also be made on the same T-DNA in tandem driven by the same promoter. These constructs can also be transformed using more than one construct, either at the same time (co-transformation) or in different transformation events.

The above-described proteins and nucleic acids may be used for producing starch or amylose in transgenic plants.

The transfer of foreign genes into the genome of an organism, in particular of a plant, is referred to as transformation.

For this purpose, methods known per se for transforming plants and regenerating plants from plant tissues or plant cells can be used, in particular in plants, for transient or stable transformation, e.g. as described in example 2.

Suitable methods for the transformation of plants are the protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method using the gene gun - also known as particle bombardment method, electroporation, the incubation of dry embryos in a DNA-containing solution, microinjection and the above-described Agrobacterium-mediated gene transfer. Said methods are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993), 128-143 and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225).

The construct to be expressed is preferably cloned into a vector which is suitable for transforming Agrobacterium tumefaciens, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711) or preferably pSUN2 (WO 02/00900).

Accordingly, the invention furthermore relates to vectors containing the above-described nucleic acids, nucleic acid constructs or expression cassettes.

Agrobacteria which have been transformed with an expression cassette can be used in a known manner for the transformation of plants, for example by bathing injured leaves or leaf sections in an agrobacteria solution and then culturing them in suitable media.

Apart from in plants, the expression cassette may also be used for transforming bacteria, in particular cyanobacteria, mosses, yeasts, filamentous fungi and algae.

Genetically modified plants, also referred to as transgenic plants herein below, are preferably prepared by cloning the fused expression cassette which expresses a amylose biosynthesis enhancing protein into a vector, for example pBin19, which is suitable for transforming Agrobacterium tumefaciens.

Agrobacteria which have been transformed with such a vector may then be used in a known manner for the transformation of plants, in particular of crop plants, for example by bathing injured leaves or leaf sections in an agrobacteria solution and then culturing them in suitable media.

The transformation of plants by agrobacteria is described, inter alia, in F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38. Transgenic plants which contain a gene for expression of a nucleic acid encoding a amylose biosynthesis enhancing protein, which has been integrated into the expression cassette, can be regenerated in a known manner from the transformed cells of the injured leaves or leaf sections.

A host plant is transformed with a nucleic acid SEQ ID NO 1 or 3 encoding an amylose biosynthesis enhancing protein by incorporating an expression cassette as insertion into a recombinant vector whose vector DNA comprises additional functional regulatory signals, for example sequences for replication or integration. Suitable vectors are described inter alia, in Methods in Plant Molecular Biology and Biotechnology (CRC Press), chapter 6/7, pp. 71-119 (1993).

By way of example, the plant expression cassette may be incorporated into a derivative of the transformation vector pBin-19 with 35s promoter (Bevan, M., Nucleic Acids Research 12: 8711-8721 (1984).

Using the above-cited recombination and cloning techniques, it is possible to clone the expression cassettes into suitable vectors for maintenance and propagation of genetic material for example in E. coli. Suitable cloning vectors are, inter alia, pBR322, pUC series, M13mp series and pACYC184. Particularly suitable are binary vectors which can replicate both in E. coli and in agrobacteria.

The invention therefore further relates to the use of the above-described nucleic acids or of the above-described nucleic acid constructs, in particular of the expression cassettes, for preparing genetically modified plants or for transforming plants, plant cells, plant tissues or parts of plants.

The use is preferably aimed at increasing the starch or amylose content of the plant, of the tubers or in other parts of the plant.

The use is most preferably aimed at increasing the starch or amylose content of wild-type or transgenic potato plants and especially the tubers of wild-type or transgenic potato plants.

Accordingly, the invention further relates to a method for preparing genetically modified plants by introducing an above-described nucleic acid or an above-described nucleic acid construct into the genome of the starting organism.

The invention further relates to the genetically modified organisms, the genetic modification increasing the activity of an amylose biosynthesis enhancing protein compared to a wild type or transgenic plant and the amylose biosynthesis enhancing protein comprising the amino acid sequence SEQ ID NO 2 or 4 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ ID NO 2 or 4.

As illustrated above, the amylose biosynthesis enhancing protein activity is increased compared to the wild type or transgenic plant preferably by increasing the gene expression of a nucleic acid encoding an amylose biosynthesis enhancing protein.

In a further preferred embodiment, gene expression of a nucleic acid encoding a amylose biosynthesis enhancing protein is increased, as illustrated above, by introducing nucleic acids encoding an amylose biosynthesis enhancing protein into the organism

and thus by over-expressing nucleic acids encoding an amylose biosynthesis enhancing protein.

Such transgenic plants, their propagation material and their plant cells, plant tissues, plant parts or tubers are a further subject of the present invention.

Genetically modified plants of the invention, which have an increased starch or amylose content and which can be consumed by humans and animals, can also be used as food- or feedstuffs or as feed and food supplements, for example directly or after processing known per se. The genetically modified plants may furthermore be used for producing starch or amylose-containing extracts of said plant and/or for producing feed and food supplements.

The invention further relates to:

I. A polynucleotide that encodes a polypeptide of SEQ ID NO 1 or 3.

II. A polynucleotide comprising at least 30 contiguous bases of SEQ ID NO 1 or 3.

III. A polynucleotide having at least 60 % sequence identity to SEQ ID NO 1 or 3, wherein the identity is based on the entire coding sequence.

IV. A polynucleotide having at least 60 % sequence identity to SEQ ID NO 1 or 3, wherein the % sequence identity is based on the entire sequence.

V. A polynucleotide which selectively hybridizes, under stringent conditions and a wash in 2 X SSC at 50 °C, to a hybridization probe derivable from the polynucleotide sequence as set forth in SEQ ID NO 1 or 3, or from the genomic sequence.

VI. A polynucleotide complementary to a polynucleotide of V.

VII. The polynucleotide of I, wherein the starch or amylose biosynthesis enhancing polynucleotide is from Solanum tuberosum.

VIII. The polynucleotide of I encoding a polypeptide, which after over-expression in a plant cell increases the starch or amylose content.

IX. The polynucleotide of I in antisense orientation, which after expression in a plant cell decreases the starch or amylose content.

X. A vector comprising at least one polynucleotide of I.

XI. An expression cassette comprising at least one polynucleotide of I operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation.

XII. A host cell which is introduced with at least one expression cassette of X.

XIII. The host cell of XI that is a plant cell.

XIV. A transgenic plant comprising at least one expression cassette of XI.

XV. The transgenic plant of XIII, wherein the plant is Solanum tuberosum.

XVI. A tuber from the transgenic plant of XIV.

XVII. An isolated protein comprising a member selected from the group consisting of:
a) a polypeptide comprising at least 10 contiguous amino acids of SEQ ID NO 2 or 4,
b) a polypeptide which is a plant amylose biosynthesis enhancing protein,
c) a polypeptide comprising at least 55 % sequence identity to SEQ ID NO 2 or 4, wherein the sequence identity is based on the entire sequence and has at least one epitope in common with an amylose biosynthesis enhancing protein.
d) a polypeptide encoded by a polynucleotide selected from SEQ ID NO 1 or 3,
e) a polypeptide of SEQ ID NO 2 or 4.

XVIII. The protein of XVII, wherein the polypeptide is catalytically active.

XIX. A ribonucleic acid sequence encoding the protein of XVIII.

XX. A method for modulating the level of amylose biosynthesise enhancing protein in a plant, comprising:
a) stably transforming a plant cell with a polynucleotide coding for an amylose biosynthesis enhancing protein operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation
b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient

to modulate the level of amylose biosynthesis enhancing protein in the plant.

XXI. The method of XX, wherein the polynucleotide coding for an amylose biosynthesis enhancing protein is selected from SEQ-ID NO 1 or 3.

XXII. The method of XX, wherein the plant is Solanum tuberosum.

XXIII. The method of XX, wherein activity of the amylose biosynthesis enhancing protein is increased.

XXIV. A method for modulating the level of starch or amylose in a plant, comprising:
a) stably transforming a plant cell with a polynucleotide coding for an amylose biosynthesis enhancing protein operably linked to a promoter, wherein the polynucleotide is in sense or anti-sense orientation,
b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate level of starch or amylose in the plant.

XXV. A method for modulating the level of amylose in a plant, comprising:
a) stably transforming a plant cell with a polynucleotide encoding an amylose biosynthesis enhancing protein operably linked to a promoter, wherein the polynucleotide is in sense or anti-sense orientation.
b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate level of amylose in the plant.

XXVI. The method of XXIV wherein the polynucleotide coding for an amylose biosynthesis enhancing protein is selected from SEQ ID NO 1 or 3.

Some of the terms used further on in the specification are defined at this point.

"Enzymatic activity/activity assay": the term enzymatic activity describes the ability of an enzyme to convert a substrate into a product. In this context, both the natural substrate of the enzyme and a synthetic modified analog of the natural substrate can be used. The enzymatic activity can be determined in what is known as an activity assay via the increase in the product, the decrease in the starting material, the decrease or increase in a specific cofactor, or a combination of at least two of the aforementioned parameters as a function of a defined period of time.

"Functional equivalents" in the present context describe nucleic acid sequences which hybridize under standard conditions with the nucleic acid sequence encoding the amylose biosynthesis enhancing protein or portions of the nucleic acid sequence encoding the amylose biosynthesis enhancing protein, and which are capable of bringing about the expression of an enzymatically active plant amylose biosynthesis enhancing protein in a cell or an organism.

It is advantageous to use short oligonucleotides of a length between 10 to 50bp, preferably 15-40bp, for example of the conserved or other regions, which can be determined via comparisons with other related genes in a manner known to the skilled worker for the hybridization. Alternatively, it is also possible to use longer fragments of the nucleic acids according to the invention or the complete sequences for the hybridization. These standard conditions vary depending on the nucleic acid used, namely oligonucleotide, longer fragment or complete sequence, or depending on which type of nucleic acid, that is DNA or RNA, is being used for the hybridization. Thus, for example, the melting temperatures for DNA:DNA hybrids are approx. 10oC lower than those of DNA:RNA hybrids of equal length. Suitable hybridization conditions are described above.

A functional equivalent is furthermore also understood as meaning, in particular, natural or artificial mutations of the relevant nucleic acid sequences of the plant amylose biosynthesis enhancing protein and their homologs from other organisms which make possible the expression of the enzymatically active plant amylose biosynthesis enhancing protein in a cell or an organism.

Thus, the scope of the present invention also extends to, for example, those nucleotide sequences which are obtained by modification of the nucleic acid sequence of a amylose biosynthesis enhancing protein. The purpose of such a modification can be, for example, the insertion of further cleavage sites for restriction enzymes, the removal of excess DNA, or the addition of further sequences. Proteins which are encoded via said nucleic acid sequences should still maintain the desired functions, despite the deviating nucleic acid sequence.

The term functional equivalent may also refer to the protein encoded by the nucleic acid sequence in question. In this case, the term functional equivalent describes a protein whose amino acid sequence is up to a specific percentage identical with that of the amylose biosynthesis enhancing protein.

Functional equivalents thus encompass naturally occurring variants of the sequences described herein, and also artificial, for example chemically synthesized, nucleic acid

sequences adapted to the codon usage, or the amino acid sequences derived therefrom.

In general, it can be said that functional equivalents independently of the amino acid sequence in question (encoded by a corresponding nucleic acid sequence) have in each case the enzymatic activity of a amylose biosynthesis enhancing protein.

"Reporter genes" encode readily quantifiable proteins. Using these genes, an assessment of transformation efficacy or of the site or time of expression can be made via growth, fluorescence, chemoluminescence, bioluminescence or resistance assay or via photometric measurement (intrinsic color) or enzyme activity. Very especially preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol. Biotechnol. 1999; 13(1):29-44) such as the "green fluorescence protein" (GFP) (Gerdes HH and Kaether C. FEBS Lett. 1996; 389(1):44-47; Chui WL et al., Curr. Biol. 1996, 6:325-330; Leffel SM et al., Biotechniques. 23(5):912-8, 1997), chloramphenicol acetyl transferase, a luciferase (Giacomin, Plant Sci. 1996, 116:59-72; Scikantha, J. Bact. 1996, 178:121; Millar et al., Plant Mol. Biol. Rep. 1992 10:324-414), and luciferase genes, in general b-galactosidase or b-glucuronidase (Jefferson et al., EMBO J. 1987, 6, 3901-3907), the Ura3 gene, the Ilv2 gene, the 2-desoxyglucose-6-phosphate phosphatase gene, b-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene, or the BASTA (= gluphosinate) resistance gene.

"Significant increase": referring to the enzymatic activity, is understood as meaning the increase in the enzymatic activity of the enzyme incubated with a candidate compound in comparison with the activity of an enzyme not incubated with the candidate compound, which lies outside an error in measurement.

"Substrate": Substrate is the compound which is recognized by the enzyme in its original function and which is converted into a product by means of a reaction catalyzed by the enzyme.

Preferably, the plant amylose biosynthesis enhancing protein is encoded by a nucleic acid sequence comprising

a) a nucleic acid sequence shown in SEQ ID NO 1 or 3; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO 2 or 4 by back translation; or

c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO 2 or 4, which has an identity with SEQ ID NO 2 or 4 of at least 50%, by back translation.

The functional equivalent of SEQ ID NO 2 or 4 set forth in c) has an identity of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57% preferably at least 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, and 70% more preferably 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85% most preferably at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID NO 2 or 4.

Example 1

Complementation study in yeast

Yeast contains two self-glycosylating proteins, Glg1p and Glg2p, which yield primers for the initiation of glycogen synthesis. For glycogen synthesis to take place in yeast it is required that either gene is functional. Yeast strain CC9, contain knock-out mutations for both genes and is therefore a null mutant regarding this specific biosynthetic function and is therefore unable to produce glycogen (Cheng, C. et al., Molecular and Cellular Biology (1995), 6632-6640). CC9 was used as a basis for complementation experiments with the isolated potato genes in order to validate their function by restoring glycogen biosynthesis in the CC9 strain. The potato genes were cloned in a yeast plasmid, pRS414 (Stratagene), and expressed with various yeast controlling elements such as Gal1, Adh1 and Glg2p promoters. CC9 was transformed by the resulting plasmids using LiCl and electroporation (Multiporator, Eppendorf). Transformed yeast colonies growing on appropriate media plates were screened by immersing in iodine solution. Wild type yeast producing glycogen is stained red brown by iodine while the null mutant CC9 is not stained. CC9 expressing the potato genes, StGH1 and StGH2, will stain red brown, when the isolated genes complement a glycogenin function in yeast and thus carry the desired function.

Example 2

Transformation method

Fully expanded leaves from in vitro propagated potato plants are diagonally cut in 2-4 pieces and precultivated on MC-plates for 2-3 days at 23-24°C.

Agrobacterium tumefaciens strain LBA4404 containing pHS1, pHS2, pHS3 or pHS4 are grown in YEB medium with 100µg rifampicin and 25µg/ml kanamycin over night on constant shaking (200 rpm) at 28°C.

The Agrobacterium culture is prepared for infection by dilution 1:20 with MS10 medium. The leaf explants are infected for 8-10 min in the bacterial solution and afterwards drained on filter paper for 5-20 seconds. The leaf segments are placed on the MS300 plates for 2 days co-cultivation under modest light at 23-24°C. At the end of co-cultivation the leaf segments are moved to M400 plates containing 400mg/l Claforan to suppress bacterial growth. After 4-5 days the explants are moved to selection medium MS400 supplemented with 400mg/l Claforan. For explants transformed with pHS1 and pHS2 50µM kanamycin was included in the media and for explants transformed with pHS3 and pHS4 0.5µM Imazamox was added to the media.
Leaf segments are transferred to fresh MS 400 selection medium every fortnight. The regenerated putative transgenic shoots are collected and cultivated on MS30 plates with 200mg/l Claforan aiming at shoot elongation.

When the shoots are 3-5 cm long, 1-2 cm are cut off and grown on microtuber medium in the dark at 25°C. After 2-5 weeks microtubers are produced. Putative transgenic plants are analysed for GUS expression in microtubers to determine the transformation efficiency.

MC plates MS300 plates with 1.5-2 ml liquid MS100 medium and covered with one sterile filter paper	MS300 4.4g/l MS-medium 2mg/l naphtyl acetic acid 1mg/l 6-benzyl amino pyridine 3% (w/v) sucrose pH 5.2
MS10 4.4 g/l MS-medium (murashige and Skoog) 1% (w/v) sucrose pH 5. 8	MS400 4.4 g/l MS-medium 2mg/l zeatine 0.01mg/l naphtyl acetic acid mg/l gibberellic acid 10% (w/v) sucrose 400 mg/l claforan 0.5 µM Imazamox or 50 µM kanamycin pH5.8

PH 5,8	Mg/l gibberellic acid 10% (w/v) sucrose 400 mg/l claforan 0,5 µM Imazamox or 50 µM kanamacin pH 5.8
µM MS30 4.4 g/l MS-medium 3% (w/v) sucrose pH 5.8	Microtuber medium 4.4 g/l MS- medium 2.5 mg/l kinetin 0.5 mg/l abscisic acid 8% sucrose 200 mg/ claforan
MS100 4.4 mg/l MS-medium 30g/l sucrose 0.5 m/lg thiamin-HCl 0.5 mg/l pyridoxin-HCl 1 mg/l nicotinacid 0.5 mg/l kinetin 29.8 mg/l ferrous sulfate hepta hydrate 1 mg/l 2,4-Dichlorophenoxyacetic acid 2 g/l caseinhydrolysate pH 5.2	

Example 3

Transgenic plant AM 99-2003

High amylose potato lines can be produced for example by using antisense, RNAi or antibody technology that target the two starch branching enzymes starch branching enzyme 1 (SBE1) and starch branching enzyme 2 (SBE2).

The high amylose potato line AM99-2003 is produced by inhibition of the starch branching enzyme activities in the mother variety Dinamo. Transformation is made with a construct of SBE1 and SBE2 in antisense orientation driven by the gbss promoter.

pBluescript containing a 1620bp fragment of the 3'end of Sbe1 between EcoRV and SpeI is cut open with SpeI (blunt) and XbaI and ligated with a 1243bp SstI (blunt) and

XbaI fragment of the 3'end of Sbe2. The Sbe2 and Sbe1 complex is cut out with EcoRV and XbaI and ligated to the SmaI and XbaI opened up binary vector pHo3.1, see figure 8. The final vector is named pHAbe12A, see figure 9 and nucleic acid sequence SEQ ID NO 15. pHo3.1 is based on pGPTVKan (Becker, D. et al., Plant Molecular Biology 20 (1992), 1195-1197) with the addition of the 987bp gbss promoter cloned at the HindIII site of pGPTVKan and the uidA gene is deleted by SmaI and SstI.

The mother variety Dinamo is transformed with the construct pHAbe12A as described in example 2.

Example 4

Down-regulation of StGH1 and StGH2 genes in potato by antisense

The StGH1 and StGH2 genes were down-regulated in potato by transformation with the genes in antisense direction in relation to a plant regulatory element. The respective antisense genes were cloned in a binary vector driven by a tuber specific gbss promoter. NptII, yielding resistance to the antibiotic kanamycin, was used as selection marker. Two varieties were transformed, Prevalent and Producent. The shoots were selected on 50 µM kanamycin, which is a standard kanamycin concentration used for potato transformation (Ooms, G et al., Theoretical and Applied Genetics 73:744-750 (1987) and Tavazza, R. et al., Plant Science 59 (1988), 175-181).

Example 5

Over-expression of StGH1 and StGH2 genes in potato

The StGH1 and StGH2 genes were over-expressed in potato driven by the tuber specific promoter gbss. A mutated AHAS gene was used as selection marker yielding tolerance to the Imazamox herbicides. Two potato varieties were transformed, Desiree and AM99-2003 a transgenic high amylose line with a 40% decrease in starch content compared to its mother variety.

Example 6

Selection of transgenic lines

Non-transgenic escapes were identified and discarded by a PCR screening method. DNA was extracted according to DNeasy 96 Plant protocol (Qiagen). In a 96 well microtiter plate, 10-15 mg leaf tissue was added to each well together with a 5mm steel

ball, each well then representing one individual shoot. The plates were frozen in N_2(l) before homogenisation. The homogenisation was done at 30Hz in a Mixermill300 for 1 min. The DNA was at the end of the extraction protocol eluted in 75µl H_2O.

Specific primers for nptII and AHAS were used for the amplification of a 246bp fragment respective a fragment of 509 bp for selection of successfully transformed lines.

Npt2_for 5'-AGCAAGGTGAGATGACAGGAGATC-3'
Npt2_rev 5'CAGACAATCGGCTGCTCTGATG-3'

AHAS1_frw: 5'-AACAACAACATCTTCTTCGATC-3'
AHAS1_rev: 5'-TAACGAGATTTGTAGCTCCG-3'.

The PCR reactions were with the extracted DNA setup and run as follow:

Reaction:

10x PCR Mix	2,0 µl
Primer frw (25µM)	0,4 µl
Primer rev (25µM)	0,4 µl
dNTPs (10mM)	0,4 µl
RedTAQ (Sigma)	1,0 µl
Templat (~20ng/µl)	4,0 µl
H_2O	11,8 µl

PCR program:

94°C	30 s	
59°C	30 s	x29 cycles
72°C	30s	
72°C	7 min	
8°C	Hold	

Example 7

Gene expression analyses

The gene expression levels of the StGH1 and StGH2 genes were analysed in the transgenic potato lines with real-time PCR (ABI prism 7900HT, Applied Biosystems). With real-time PCR the change of gene expression can be analysed regarding RNA

expression levels. For pHS1 and pHS2 transgenic lines, expression of both sense and antisense RNA of StGH1 and StGH2 was measured, while in pHS3 and pHS4 transgenic lines the change in StGH1 and StGH2 mRNA expression was analysed. The target for pHS1 and pHS2 is to reduce transcript levels of StGH1 and StGH2 respectively while the target for pHS3 and pHS4 is to increase transcript levels of the respective genes.

RNA was isolated from microtubers of the transgenic potato lines and mother varieties using Invisorb Spin Plant-RNA mini kit (Invitek). A reverse transcription reaction was made with 250 ng total RNA in 25µl total reaction volume using TaqMan reverse transcription reagents (Applied Biosystems). Separate and specific primers (see table 1) were designed and used for the reverse transcription reaction in order to be able to differentiate the endogenous expression from the antisense RNA expression of the respective genes.

StGH1 sense RNA	5´-TGAAGACAGCACAAAACTGG-3´
StGH1 antisense RNA	5´-GTGAAAGTTTGAACGCACAC-3´
StGH2 sense RNA	5´-AGTGCCATAACATGCTTTCC -3´
StGH2 antisense RNA	5´-CACATTTCAGCTGTTGATGGA-3´

Table 1

5µl of the reverse transcription reaction was used in triplicate analyses together with specific sequence detection primers, TaqMan MGB probe (see table 2) and UMM mastermix (Applied Biosystems) and determined with real-time PCR.

StGH1
Forward Primer: TCGAGTCGCCACGTAGAACTC Reverse primer: GAAATGCGTATGCGACTATGATG TaqMan probe: AGTCTCTCGGAGTTCC
StGH2
Forward primer: GGTGCTGATCCTCCAGTTCTCT Reverse primer: GTCCCTGAAGCATAACCAAGGT TaqMan probe: TTCTGCACTACTTAGGCCT

Table 2

Down-regulation of the two genes resulted in a decrease in gene expression in transgenic lines compared to their mother varieties in the order of 50-95 %.

Over-expression of the two genes resulted in a 2-10 times increase in gene expression in transgenic lines compared to their mother varieties.

Ref no	Construct	Variety	Δ Ct s	Times increase or decrease in gene expression compared to mother variety
P01-041-84	pHS1	Producent	-1,14	-1,3
P02-325-1	pHS1	Producent	-2,03	-4,1
P02-325-9	pHS1	Producent	-1,47	-2,2
P02-325-11	pHS1	Producent	-1,25	-1,6
P02-325-15	pHS1	Producent	-2,52	-6,3
P02-325-25	pHS1	Producent	-2,0	-4
P02-325-27	pHS1	Producent	-1,64	-2,7
P02-325-33	pHS1	Producent	-1,59	-2,5
P02-325-34	pHS1	Producent	-1,52	-2,3
P02-325-63	pHS1	Producent	-1,53	-2,3
P02-300-37	pHS2	Prevalent	-1,27	-1,6
P02-300-66	pHS2	Prevalent	-1,04	-1,1
P02-300-71	pHS2	Prevalent	-1,13	-1,3
P02-300-73	pHS2	Prevalent	-1,1	-1,2
P02-300-80	pHS2	Prevalent	-2,12	-4,5
P02-300-127	pHS2	Prevalent	-1,67	-2,8
P02-300-140	PHS2	Prevalent	-3,96	-15,7
P02-303-31	pHS2	Prevalent	-1,16	-1,4
P02-303-64	pHS2	Prevalent	-1,15	-1,3
P02-305-54	pHS2	Prevalent	-1,33	-1,8
P02-320-24	pHS2	Prevalent	-1,03	-1,1
P02-307-4	pHS3	Desirée	1,82	3,3
P02-307-5	pHS3	Desirée	2,68	7,2
P02-307-12	pHS3	Desirée	2,67	7,1
P02-307-14	pHS3	Desirée	1,83	3,3
P02-307-15	pHS3	Desirée	1,79	3,2
P02-307-33	pHS3	Desirée	3,21	10,3
P02-307-43	pHS3	Desirée	2,7	7,3
P02-307-51	pHS3	Desirée	2,73	7,5
P02-307-80	pHS3	Desirée	2,78	7,7
P02-307-87	pHS3	Desirée	1,02	1,1

Ref no	Construct	Variety	Δ Ct s	Times increase or decrease in gene expression compared to mother variety
P02-307-148	pHS3	Desirée	1,88	3,5
P02-309-63	pHS3	AM99-2003	1,64	2,7
P02-309-106	pHS3	AM99-2003	1,75	3,1
P02-311-59	pHS3	AM99-2003	1,17	1,4
P02-312-15	pHS4	AM99-2003	1,03	1,1
P02-313-21	pHS4	AM99-2003	1,54	2,4

Table 3: Gene expression analyses based on Real- Time PCR

Example 8

Dry matter analysis

Dry matter has been analysed on microtubers from transgenic lines transformed with pHS1, pHS2, pHS3 and pHS4 showing a down-regulation or over expression of the genes. Since starch normally contribute to more than 80% of the dry matter in potato tubers, an increase or decrease in starch content will affect also the dry matter content.

Two microtubers of each line were harvested when they had reached maturity. Dry matter was calculated for mature microtubers weighed before and after 72 hours drying at 60°C. For comparison microtubers from the varieties Dinamo, Desiree, Prevalent, Producent and P737 with starch contents between 13 and 28% (when grown in field) were used. The starch content of microtubers is not as high as starch content of field grown tubers. However dry matter content can readily be compared in microtubers and that value is well correlated to the determined starch content in field grown tubers. In table 4 the average dry matter for the different varieties, calculated on ten or more microtubers, is shown.

Variety	Starch content field grown tubers	Dry matter microtubers
Dinamo	13%	14,8
Desirée	16%	16,1
Producent	22%	19,2
Prevalent	22%	19,7
P737	28%	21,6

Table 4. Dry matter content of 5 varieties based on 10 or more microtubers

One of each pHS1 and pHS2 with confirmed decrease in gene-expression have been analyzed for dry matter so far. Those two have a decrease in dry matter of 7 and 11% compared to their mother varieties.

For the pHS3 lines 8 of 9 of the confirmed over-expressed lines show an increase of up to 36% in dry matter. See table 5.

Ref No	Construct	Variety	Dry matter in relation to mother variety (%)
41-84	pHS1	Producent	89
300-127	pHS2	Prevalent	93
300-140	PHS2	Prevalent	96
307-4	pHS3	Desirée	106
307-5	pHS3	Desirée	117
307-15	pHS3	Desirée	124
307-33	pHS3	Desirée	116
307-57	pHS3	Desirée	136
309-63	pHS3	AM99-2003	134
309-106	pHS3	AM99-2003	109
309-111	pHS3	AM99-2003	108

Table 5. Dry matter content on transgenic lines with confirmed down-regulation or over expression of the StGH1 and StGH2 genes

Example 9

Starch content analysis

For analysis of starch content a total starch assay procedure from Megazyme International Ireland Ltd., Bray, Co.Wicklow, Ireland (AOAC Method 996.1; AACC method 76.13; ICC standard method No. 168) was used according to the suppliers instructions.

Starch content was analysed on microtubers from all transgenic lines transformed with pHS1, pHS2, pHS3 and pHS4. The microtubers were harvested when they had reached maturity. Mature microtubers were ground and maltosaccharides and free glucose residues were washed away with ethanol. The microtuber starch was treated with DMSO to ensure the complete solubilisation of samples with high levels of resistant starch, as the high amylose clones.

Samples were analysed with a standard spectrophotometric assay procedure. The transgenic lines were compared to potato varieties with known starch content ranging from 8% to 30%. The results give an indication on the change in starch content related to the genetic modification of the different transgenic lines.

Example 10

Field-testing of transgenic potato lines

Produced transgenic lines are tested in field trials for determination of agronomic performance in relation to mother variety and other varieties used for starch production. Starch content, which is a main agronomic factor of importance for crops used for starch processing, can measured by several different methods.

Under water weighing is performed on a scale in a tub of water and where an increase in starch content is associated with an increase in density of the sample.
An increase starch is associated with an increased dry matter content, which can be measured by comparing the tissue fresh weight to tissue dry weight after extensive water elimination in an oven.

Starch content can also be measured by enzymatic methods as described under starch content analyses.

Example 11

Increased amylose and starch yield in potatoes

Potato varieties used for starch production as well as genotypes with a high amylose content are transformed with gene constructs as described above for the over-expression of a starch biosynthesis enhancing protein. The over-expression of StGH1 or StGH2 in potato plants will result in an increased amylose content of the transgenic plant compared to the starting plant.

Example 12

Increased solids and improved processing quality of potatoes

In another aspect the invention may be used to increase the solids content of varieties that are used for processed potato products or table potato varieties. The potato genotypes are transformed with gene constructs as described above for the over-expression

of a starch biosynthesis enhancing protein. This starch biosynthesis enhancing protein may be derived from genes described above or other plant genes containing the same functional domains.

What is claimed:

1. A method of increasing the production of starch in plants comprising culturing a plant with enhanced expression or activity of at least one starch biosynthesis enhancing protein.

2. The method of claim 1, wherein said starch has a high amylose content.

3. The method as claimed in either claim 1 or 2, wherein production of amylose is increased.

4. The method as claimed in any one of claims 1 to 3, wherein said method comprises over-expression of an amylose biosynthesis enhancing protein.

5. The method as claimed in claim 4, wherein said protein comprises the SEQ ID NO: 2 or 4 or a protein derived from this sequence by substitution, insertion or deletion of amino acids and which has at least 50% identity at the amino acid level with SEQ ID NO: 2 or 4.

6. The method as claimed in any of claims 1 to 5, wherein the amylose biosynthesis enhancing protein is encoded by a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence comprising a nucleotide sequence which is at least 60% identical to the nucleic acid sequence of SEQ ID NO: 1 or 3;

b) a nucleic acid sequence comprising a fragment of at least 30 nucleotides of a nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO:1 or 3;

c) a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2 or 4 and

d) a nucleic acid sequence which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or 4 or wherein the fragment comprises at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2 or 4,

7. The method as claimed in any one of claims 1 to 6, wherein the amylose biosynthesis enhancing protein is encoded by a nucleic acid sequence comprising the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3.

8. The method as claimed in any one of claims 1 to 7, wherein deficiency or decreased activity is achieved by a method selected from the group consisting of:

a) knock-out of the gene encoding said protein;

b) mutagenesis of the gene encoding said protein, wherein said mutation can be induced in the coding, non-coding, or regulatory regions of said gene;

c) expression of an anti-sense RNA, wherein said anti-sense RNA is complementary to at least part of the RNA encoding said protein;

9. A method of producing amylose type starch by culturing a plant which over-expresses SEQ ID NO:1 or 3 or has increased amylose biosynthesis enhancing activity under conditions such that the plant produces an increased amount of amylose type starch.

10. The method of any of the preceeding claims, wherein said plant belongs to the genus Solanum.

11. The method of claim 10, wherein said plant is Solanum tuberosum.

12. A nucleic acid sequence SEQ ID NO:1 encoding an amylose biosynthesis enhancing protein.

13. A nucleic acid sequence SEQ ID NO:3 encoding an amylose biosynthesis enhancing protein.

14. An amino acid sequence SEQ ID NO:2 having amylose biosynthesis enhancing activity.

15. An amino acid sequence SEQ ID NO:4 having amylose biosynthesis enhancing acitivity.

16. A transgenic expression cassette comprising in combination with a regulatory sequence a nucleic acid sequence selected from the group consisting of:

a) a nucleic acid sequence comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3,

b) a nucleic acid sequence comprising a fragment of at least 30 nucleotides of a nucleic acid sequence comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3,

c) a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or

d) a nucleic acid sequence which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 wherein the fragment comprises at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4

wherein said regulatory sequence is capable of mediating expression of said nucleic acid sequence in a plant.

17. A transgenic expression cassette of claim 16, wherein said regulatory sequence is a promoter sequence heterologous with regard to said nucleic acid sequence.

18. A transgenic expression cassette of claim 16, wherein said regulatory sequence is a tuber specific promoter sequence.

19. A transgenic expression cassette of either claim 16, 17 or 18, wherein said nucleic acid sequence is arranged in antisense or sense orientation with regard to said promoter sequence.

20. A transgenic expression cassette of any of the claims 16 to 19, wherein said nucleic acid sequence encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4.

21. A transgenic expression cassette of any of the claims 16 to 20, wherein said nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3.

22. A transgenic expression cassette of any of the claims 16 to 21, wherein said nucleic acid sequence encodes a naturally occurring variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4.

23. A transgenic host cell transformed with an expression cassette of any of the claims 16 to 22.

24. A transgenic host cell of claim 23, wherein said host cell belongs to the genus Solanum.

25. A transgenic plant comprising an expression cassette of any of claims 16 to 22.

26. A transgenic potato plant comprising an expression cassette of any of claims 16 to 22.

27. A transgenic potato plant, plant part, seed or tuber comprising an expression cassette of any of claims 16 to 22.

SEQUENCE LISTING

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<141> 2003-03-07

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Glu Met Ile Ala Val Leu Ile Gln Ser Ile Phe Tyr Leu Gly Ile Ile
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305                 310                 315                 320

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465

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ttcagtctgt gctcttaatt agaatggaat tctgtgagtg ggtttagaat tgggaggatg 1866

ttttgtgttg tacatggact atctctggtc tcttgaatgc tactccagga aaaagattgt 1926

ttctcactta atttttttctg ttactaaatt gtatgtggaa taggttcttt aaaatttatt 1986

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Ala Tyr Ala Thr Met Met Tyr Met Gly Thr Pro Arg Asp Tyr Glu Phe
35 40 45

Tyr Val Ala Thr Arg Val Met Leu Arg Ser Leu Thr Arg Leu Gly Val
50 55 60

Glu Ala Asp Leu Val Val Ile Ala Ser Leu Asp Val Pro Leu Arg Trp

65 70 75 80

Val Gln Thr Leu Glu Gln Glu Asp Gly Ala Lys Val Val Arg Val Lys
85 90 95

Asn Leu Asn Asn Pro Tyr Cys Ile Asn Pro Asn Trp Arg Phe Lys Leu
100 105 110

Thr Leu Asn Lys Leu Tyr Ala Trp Ser Leu Val Asn Tyr Asp Arg Val
115 120 125

Val Met Leu Asp Ala Asp Asn Leu Phe Leu Gln Lys Thr Asp Glu Leu
130 135 140

Phe Gln Cys Gly Gln Phe Cys Ala Val Phe Ile Asn Pro Cys Ile Phe
145 150 155 160

His Thr Gly Leu Phe Val Leu Gln Pro Ser Lys Lys Val Phe Asn Asp
165 170 175

Met Ile His Glu Ile Glu Ile Gly Arg Glu Asn Gln Asp Gly Ala Asp
180 185 190

Gln Gly Phe Ile Gly Gly His Phe Pro Asp Leu Leu Asp Arg Pro Met
195 200 205

Phe His Pro Pro Leu Asn Gly Thr Gln Leu Gln Gly Ser Tyr Arg Leu
210 215 220

Pro Leu Gly Tyr Gln Met Asp Ala Ser Tyr Tyr Tyr Leu Lys Leu His
225 230 235 240

Trp Ser Val Pro Cys Gly Pro Asn Ser Val Ile Thr Phe Pro Gly Ala
245 250 255

Pro Trp Leu Lys Pro Trp Tyr Trp Trp Ser Trp Pro Val Leu Pro Leu
260 265 270

Gly Ile Gln Trp His Glu Gln Arg Arg Leu Thr Val Gly Tyr Gly Ala
275 280 285

Glu Met Ile Ala Val Leu Ile Gln Ser Ile Phe Tyr Leu Gly Ile Ile
290 295 300

Ala Val Thr Arg Leu Ala Arg Pro Asn Leu Ser Lys Leu Cys Tyr Arg
305 310 315 320

His Asp Asp Ser Lys Ser Ala Phe Leu Leu Arg Thr Gly Leu Lys Leu
325 330 335

Ile Ala Ile Trp Ser Ile Leu Ala Ala Tyr Thr Val Pro Tyr Phe Val
340 345 350

Ile Pro Cys Thr Val His Pro Leu Val Gly Trp Ser Leu Tyr Leu Leu
355 360 365

Gly Ser Phe Ser Leu Ser Cys Ile Thr Val Asn Ala Phe Leu Leu Pro
370 375 380

Met Leu Pro Val Leu Val Pro Trp Ile Gly Ile Leu Gly Ala Leu Leu
385 390 395 400

Val Met Ala Tyr Pro Trp Tyr Asn Asp Gly Val Val Arg Ala Met Ala
405 410 415

Val Phe Thr Tyr Ala Phe Cys Ala Ser Pro Ala Leu Trp Met Ala Leu
420 425 430

Val Lys Ile Lys Cys Ser Leu His Val Ser Leu Glu Arg Glu Gly Phe
435 440 445

Leu Pro Lys Ile Ser Glu Ser Thr Ala Pro Ala Gly Ser Asn Lys Leu
450 455 460

Tyr
465

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acttgaaaaa ggaaagccca ga atg aga gga agt tta gct ggt gga cca cct 172
Met Arg Gly Ser Leu Ala Gly Gly Pro Pro
1 5 10

agt cct att gaa cct aga cag agg ctt tct gta ttc act gag gaa aca 220
Ser Pro Ile Glu Pro Arg Gln Arg Leu Ser Val Phe Thr Glu Glu Thr
15 20 25

agc aaa aga agg ttc ttg aga agt aaa gtt ttc aga gat ggg gag aga 268
Ser Lys Arg Arg Phe Leu Arg Ser Lys Val Phe Arg Asp Gly Glu Arg
30 35 40

gct ctt cat agt ccc acc aaa aac agg aat ttt acc tgc aag ttc cca 316
Ala Leu His Ser Pro Thr Lys Asn Arg Asn Phe Thr Cys Lys Phe Pro
45 50 55

act gtg aag ctt ata ttg ggt gtt att gct ctg gtt gca att tgg tca 364
Thr Val Lys Leu Ile Leu Gly Val Ile Ala Leu Val Ala Ile Trp Ser
60 65 70

ctc tgg cat tct cca gca att tat aac acg gaa tac ata tct agt tca 412
Leu Trp His Ser Pro Ala Ile Tyr Asn Thr Glu Tyr Ile Ser Ser Ser
75 80 85 90

ggc tct cgg gct gct ttg atg cac aga gag tta agt ggt cat tct tca 460
Gly Ser Arg Ala Ala Leu Met His Arg Glu Leu Ser Gly His Ser Ser
95 100 105

gct gat caa cgt tat aca tca ctt tta gat att gac tgg gac caa att 508
Ala Asp Gln Arg Tyr Thr Ser Leu Leu Asp Ile Asp Trp Asp Gln Ile
110 115 120

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tcc caa gtt att gag aaa ctg gcc gat agg cat gag tat cag ggc gta   556
Ser Gln Val Ile Glu Lys Leu Ala Asp Arg His Glu Tyr Gln Gly Val
        125                 130                 135

ggg ata tta aac ttc aat gac agt gaa att gat cag ttg aag gag tta   604
Gly Ile Leu Asn Phe Asn Asp Ser Glu Ile Asp Gln Leu Lys Glu Leu
    140                 145                 150

cta ccg gac gct gag cat gta atc ttg aac ctg gat cac gtc ccg aat   652
Leu Pro Asp Ala Glu His Val Ile Leu Asn Leu Asp His Val Pro Asn
155                 160                 165                 170

aat ata aca tgg gaa aca ata tat cct gaa tgg ata gat gaa gaa gaa   700
Asn Ile Thr Trp Glu Thr Ile Tyr Pro Glu Trp Ile Asp Glu Glu Glu
                175                 180                 185

gaa ttt gag gtc ccc act tgt cct tct ctg ccc aaa att cag ttt ccg   748
Glu Phe Glu Val Pro Thr Cys Pro Ser Leu Pro Lys Ile Gln Phe Pro
            190                 195                 200

ggt aaa cca agg att gat ctc ata gtt gta aag ctt cca tgc aag aag   796
Gly Lys Pro Arg Ile Asp Leu Ile Val Val Lys Leu Pro Cys Lys Lys
        205                 210                 215

tct aag gac tgg tat aga gat gta gct cgt ttt cac ttg cag ctg gca   844
Ser Lys Asp Trp Tyr Arg Asp Val Ala Arg Phe His Leu Gln Leu Ala
    220                 225                 230

gca gca agg ctg gct gcc agt aat aaa ggg tat cat cca ata cat gtg   892
Ala Ala Arg Leu Ala Ala Ser Asn Lys Gly Tyr His Pro Ile His Val
235                 240                 245                 250

ctt ctg gtt act gag cat ttc cca acc ccc aat ctg ttc acc tgt aaa   940
Leu Leu Val Thr Glu His Phe Pro Thr Pro Asn Leu Phe Thr Cys Lys
                255                 260                 265

gag tta gtt gta cgt gaa ggc aat gca tgg cta tat gaa cct aat ctg   988
Glu Leu Val Val Arg Glu Gly Asn Ala Trp Leu Tyr Glu Pro Asn Leu
            270                 275                 280

aac act tta aga gag aag ctc cac ctc cct gtt ggg tca tgt gaa ctt  1036
Asn Thr Leu Arg Glu Lys Leu His Leu Pro Val Gly Ser Cys Glu Leu
        285                 290                 295

gca gtt cct ctc aag gct aaa gca aat tgg cac tct gga aat gta aga  1084
Ala Val Pro Leu Lys Ala Lys Ala Asn Trp His Ser Gly Asn Val Arg
    300                 305                 310

cga gaa gcc tat gca act att ctc cac tca gca aat ttt tat gta tgt  1132
Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Asn Phe Tyr Val Cys
315                 320                 325                 330

gga gcc ata gct gca gca cag agt att cgc ttg gca ggt tca acc cga  1180
Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Leu Ala Gly Ser Thr Arg
                335                 340                 345

gat ctt gtg ata ctt gtt gat gag act atc agt gac tac cac agg ggt  1228
Asp Leu Val Ile Leu Val Asp Glu Thr Ile Ser Asp Tyr His Arg Gly
            350                 355                 360

ggt tta gag gct gcc gga tgg aag atc cac acg ata aag aga ata agg  1276
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Gly Leu Glu Ala Ala Gly Trp Lys Ile His Thr Ile Lys Arg Ile Arg
365                 370                 375

aat cct aaa gct gaa cag gat gcc tac aat gag tgg aac tat agc aaa   1324
Asn Pro Lys Ala Glu Gln Asp Ala Tyr Asn Glu Trp Asn Tyx Ser Lys
380                 385                 390

ttt cgt ctc tgg cag ctg aca gat tat gac aaa atc atc ttc att gat   1372
Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp
395                 400                 405                 410

gcg gat ttg ttg ata ctg aga aat att gat ttt ctc ttt gag atg cct   1420
Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Glu Met Pro
                415                 420                 425

gaa ata act gca ata gga aat aat gca acc ctt ttt aat tca ggc gtg   1468
Glu Ile Thr Ala Ile Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val
            430                 435                 440

atg gtc gtt gaa cca tca aat tgc aca ttt cag ctg ttg atg gat cat   1516
Met Val Val Glu Pro Ser Asn Cys Thr Phe Gln Leu Leu Met Asp His
        445                 450                 455

atc aat gag att gaa tca tac aat ggt ggg gat cag ggg tat ttg aac   1564
Ile Asn Glu Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn
    460                 465                 470

gaa ata ttc acc tgg tgg cat agg atc cca aaa cac atg aac ttt ttg   1612
Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe Leu
475                 480                 485                 490

aaa cat tat tgg gaa ggt gat gag gag gag aag aag caa atg aaa aca   1660
Lys His Tyr Trp Glu Gly Asp Glu Glu Glu Lys Lys Gln Met Lys Thr
                495                 500                 505

cgt ctt ttt ggt gct gat cct cca gtt ctc tat gtt ctg cac tac tta   1708
Arg Leu Phe Gly Ala Asp Pro Pro Val Leu Tyr Val Leu His Tyr Leu
            510                 515                 520

ggc ctg aaa cct tgg tta tgc ttc agg gac tac gat tgc aac tgg aat   1756
Gly Leu Lys Pro Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn
        525                 530                 535

gtg ggt aag ttg cag gag ttt gca agt gat gtg gca cac agg acg tgg   1804
Val Gly Lys Leu Gln Glu Phe Ala Ser Asp Val Ala His Arg Thr Trp
    540                 545                 550

tgg aag gtc cat gat gcc atg cca gat aac tta cat aaa tat tgt ttg   1852
Trp Lys Val His Asp Ala Met Pro Asp Asn Leu His Lys Tyr Cys Leu
555                 560                 565                 570

ctt agg tct aaa cag aag gct gca cta gag tgg gat cga aga gaa gct   1900
Leu Arg Ser Lys Gln Lys Ala Ala Leu Glu Trp Asp Arg Arg Glu Ala
                575                 580                 585

gag aaa gct aac ttt tca gat ggt cat tgg aag atc aaa ata aag gac   1948
Glu Lys Ala Asn Phe Ser Asp Gly His Trp Lys Ile Lys Ile Lys Asp
            590                 595                 600

cca cgt ttg gag act tgt tat gaa gaa ttt tgc ttc tgg gaa agc atg   1996
Pro Arg Leu Glu Thr Cys Tyr Glu Glu Phe Cys Phe Trp Glu Ser Met
        605                 610                 615
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tta tgg cac tgg ggt gaa aca aac tgg aca gat aat gcc acc tct tca 2044
Leu Trp His Trp Gly Glu Thr Asn Trp Thr Asp Asn Ala Thr Ser Ser
620 625 630

cca aca cct ccc atg gtc aat act gct tca ctt tct tct ttg 2086
Pro Thr Pro Pro Met Val Asn Thr Ala Ser Leu Ser Ser Leu
635 640 645

taactaggag gttctgttac tatacctcgc tagtctgtaa gtaatacaga gtcacaactt 2146

gaatgtcaac cagtgccatt agtatacatg gtgtcaacac tttccccacc cttgaaaaaa 2206

aaaaaaaaaa aaaaaaaaaa aaaa 2230

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Gln Arg Leu Ser Val Phe Thr Glu Glu Thr Ser Lys Arg Arg Phe Leu
20 25 30

Arg Ser Lys Val Phe Arg Asp Gly Glu Arg Ala Leu His Ser Pro Thr
35 40 45

Lys Asn Arg Asn Phe Thr Cys Lys Phe Pro Thr Val Lys Leu Ile Leu
50 55 60

Gly Val Ile Ala Leu Val Ala Ile Trp Ser Leu Trp His Ser Pro Ala
65 70 75 80

Ile Tyr Asn Thr Glu Tyr Ile Ser Ser Ser Gly Ser Arg Ala Ala Leu
85 90 95

Met His Arg Glu Leu Ser Gly His Ser Ser Ala Asp Gln Arg Tyr Thr
100 105 110

Ser Leu Leu Asp Ile Asp Trp Asp Gln Ile Ser Gln Val Ile Glu Lys
115 120 125

Leu Ala Asp Arg His Glu Tyr Gln Gly Val Gly Ile Leu Asn Phe Asn
130 135 140

Asp Ser Glu Ile Asp Gln Leu Lys Glu Leu Leu Pro Asp Ala Glu His
145 150 155 160

Val Ile Leu Asn Leu Asp His Val Pro Asn Asn Ile Thr Trp Glu Thr
165 170 175

Ile Tyr Pro Glu Trp Ile Asp Glu Glu Glu Glu Phe Glu Val Pro Thr
180 185 190

Cys Pro Ser Leu Pro Lys Ile Gln Phe Pro Gly Lys Pro Arg Ile Asp
195 200 205

Leu Ile Val Val Lys Leu Pro Cys Lys Lys Ser Lys Asp Trp Tyr Arg

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      210                 215                 220

Asp Val Ala Arg Phe His Leu Gln Leu Ala Ala Ala Arg Leu Ala Ala
225                 230                 235                 240

Ser Asn Lys Gly Tyr His Pro Ile His Val Leu Leu Val Thr Glu His
                245                 250                 255

Phe Pro Thr Pro Asn Leu Phe Thr Cys Lys Glu Leu Val Val Arg Glu
            260                 265                 270

Gly Asn Ala Trp Leu Tyr Glu Pro Asn Leu Asn Thr Leu Arg Glu Lys
        275                 280                 285

Leu His Leu Pro Val Gly Ser Cys Glu Leu Ala Val Pro Leu Lys Ala
    290                 295                 300

Lys Ala Asn Trp His Ser Gly Asn Val Arg Arg Glu Ala Tyr Ala Thr
305                 310                 315                 320

Ile Leu His Ser Ala Asn Phe Tyr Val Cys Gly Ala Ile Ala Ala Ala
                325                 330                 335

Gln Ser Ile Arg Leu Ala Gly Ser Thr Arg Asp Leu Val Ile Leu Val
            340                 345                 350

Asp Glu Thr Ile Ser Asp Tyr His Arg Gly Gly Leu Glu Ala Ala Gly
        355                 360                 365

Trp Lys Ile His Thr Ile Lys Arg Ile Arg Asn Pro Lys Ala Glu Gln
    370                 375                 380

Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu
385                 390                 395                 400

Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu
                405                 410                 415

Arg Asn Ile Asp Phe Leu Phe Glu Met Pro Glu Ile Thr Ala Ile Gly
            420                 425                 430

Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Val Glu Pro Ser
        435                 440                 445

Asn Cys Thr Phe Gln Leu Leu Met Asp His Ile Asn Glu Ile Glu Ser
    450                 455                 460

Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp Trp
465                 470                 475                 480

His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Tyr Trp Glu Gly
                485                 490                 495

Asp Glu Glu Glu Lys Lys Gln Met Lys Thr Arg Leu Phe Gly Ala Asp
            500                 505                 510

Pro Pro Val Leu Tyr Val Leu His Tyr Leu Gly Leu Lys Pro Trp Leu
        515                 520                 525

Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Gly Lys Leu Gln Glu
    530                 535                 540
```

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Phe Ala Ser Asp Val Ala His Arg Thr Trp Trp Lys Val His Asp Ala
545                 550                 555                 560

Met Pro Asp Asn Leu His Lys Tyr Cys Leu Leu Arg Ser Lys Gln Lys
                565                 570                 575

Ala Ala Leu Glu Trp Asp Arg Arg Glu Ala Glu Lys Ala Asn Phe Ser
            580                 585                 590

Asp Gly His Trp Lys Ile Lys Ile Lys Asp Pro Arg Leu Glu Thr Cys
        595                 600                 605

Tyr Glu Glu Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp Gly Glu
    610                 615                 620

Thr Asn Trp Thr Asp Asn Ala Thr Ser Ser Pro Thr Pro Pro Met Val
625                 630                 635                 640

Asn Thr Ala Ser Leu Ser Ser Leu
                645
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Met Asp Leu Gln Arg Thr Leu Met Phe Ser Cys Trp Val Leu Ser Leu
  1               5                  10                  15

ttg atc atc aaa acg aca gcg tat aac gag aaa cag ctg ttc cag ccg   96
Leu Ile Ile Lys Thr Thr Ala Tyr Asn Glu Lys Gln Leu Phe Gln Pro
            20                  25                  30

ctt gaa acg gaa aac gca aac gcg atg acc gcg gtt atg gag cga gga   144
Leu Glu Thr Glu Asn Ala Asn Ala Met Thr Ala Val Met Glu Arg Gly
        35                  40                  45

tta aag acg cag cgg cgg ccg gag cac aag aac gct tat gcg acg atg   192
Leu Lys Thr Gln Arg Arg Pro Glu His Lys Asn Ala Tyr Ala Thr Met
    50                  55                  60

atg tac atg gga aca cca aga gac tac gag ttc tac gtt gcg aca cgt   240
Met Tyr Met Gly Thr Pro Arg Asp Tyr Glu Phe Tyr Val Ala Thr Arg
65                  70                  75                  80

gtc ttg atc aga tcg ctt aag agt ctc cac gtg gac gct gat atc gtc   288
Val Leu Ile Arg Ser Leu Lys Ser Leu His Val Asp Ala Asp Ile Val
                85                  90                  95

gtt ata gcc tcc ctc gac gtt cct atc aac tgg att cac gct ctg gaa   336
Val Ile Ala Ser Leu Asp Val Pro Ile Asn Trp Ile His Ala Leu Glu
            100                 105                 110

gaa gaa gat gga gct aaa gta gtg aga gta gag aat ctt gag aat cca   384
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Glu Glu Asp Gly Ala Lys Val Val Arg Val Glu Asn Leu Glu Asn Pro
        115                 120                 125

tac aag aaa caa acc aac ttc gac aac aga ttc aag ctt agt cta aac   432
Tyr Lys Lys Gln Thr Asn Phe Asp Asn Arg Phe Lys Leu Ser Leu Asn
    130                 135                 140

aag ctc tac gct tgg tct ctc tct gat tat gac cgt gtt gta atg ctt   480
Lys Leu Tyr Ala Trp Ser Leu Ser Asp Tyr Asp Arg Val Val Met Leu
145                 150                 155                 160

gat gtc gac aat ctc ttt ctc aag aac acc gac gag ctc ttc cag tgt   528
Asp Val Asp Asn Leu Phe Leu Lys Asn Thr Asp Glu Leu Phe Gln Cys
                165                 170                 175

ggc caa ttt tgt gct gtc ttc atc aac cct tgc atc ttc cac act ggt   576
Gly Gln Phe Cys Ala Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly
            180                 185                 190

ctc ttt gtg ttg cag cca tca atg gag gtc ttt aga gac atg ctt cat   624
Leu Phe Val Leu Gln Pro Ser Met Glu Val Phe Arg Asp Met Leu His
        195                 200                 205

gag ctt gaa gta aag aga gat aac cct gat gga gct gat caa ggc ttt   672
Glu Leu Glu Val Lys Arg Asp Asn Pro Asp Gly Ala Asp Gln Gly Phe
    210                 215                 220

ctt gtc agc tac ttc tct gat tta ctc aat cag cct ctc ttt cgt cct   720
Leu Val Ser Tyr Phe Ser Asp Leu Leu Asn Gln Pro Leu Phe Arg Pro
225                 230                 235                 240

cct ccc gat aac cgc acc gcg ctt aag gga cat ttt agg ctt cct ttg   768
Pro Pro Asp Asn Arg Thr Ala Leu Lys Gly His Phe Arg Leu Pro Leu
                245                 250                 255

gga tat caa atg gac gca tct tat tac tac ctt aag ctc aga tgg aac   816
Gly Tyr Gln Met Asp Ala Ser Tyr Tyr Tyr Leu Lys Leu Arg Trp Asn
            260                 265                 270

gta cca tgt gga cca aac agt gtg ata acg ttc cca gga gca gta tgg   864
Val Pro Cys Gly Pro Asn Ser Val Ile Thr Phe Pro Gly Ala Val Trp
        275                 280                 285

tta aag cca tgg tat tgg tgg tca tgg cct gtt ctt cct tta ggc ctt   912
Leu Lys Pro Trp Tyr Trp Trp Ser Trp Pro Val Leu Pro Leu Gly Leu
    290                 295                 300

tca tgg cac cac caa cgc cgc tac acg att agt tat tca gca gag atg   960
Ser Trp His His Gln Arg Arg Tyr Thr Ile Ser Tyr Ser Ala Glu Met
305                 310                 315                 320

cct tgg gtc cta acc caa gca gtg ttc tac cta gga att ata cta gtc  1008
Pro Trp Val Leu Thr Gln Ala Val Phe Tyr Leu Gly Ile Ile Leu Val
                325                 330                 335

aca cgt cta gcg cgt ccc aac atg acc aag cta tgt tac cga cgt tct  1056
Thr Arg Leu Ala Arg Pro Asn Met Thr Lys Leu Cys Tyr Arg Arg Ser
            340                 345                 350

gat aag aat cta agc atg atc cag aca gct ttc aag ttt gtt gca ctc  1104
Asp Lys Asn Leu Ser Met Ile Gln Thr Ala Phe Lys Phe Val Ala Leu
        355                 360                 365
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ctc ttt atc ctc tca gcc tac att ata cca ttc ttc atc atc cca cag   1152
Leu Phe Ile Leu Ser Ala Tyr Ile Ile Pro Phe Phe Ile Ile Pro Gln
    370                 375                 380

acg atc cac ccg ctc att ggt tgg tct ctc tac tta acc ggc tcc ttt   1200
Thr Ile His Pro Leu Ile Gly Trp Ser Leu Tyr Leu Thr Gly Ser Phe
385                 390                 395                 400

gct ctc tct acc ata ccc atc aac gcc ttc ttg ctt ccc att ctc cct   1248
Ala Leu Ser Thr Ile Pro Ile Asn Ala Phe Leu Leu Pro Ile Leu Pro
                405                 410                 415

gtc ata aca ccg tgg ctt ggc att ttc ggg aca ctc ctc gtg atg gct   1296
Val Ile Thr Pro Trp Leu Gly Ile Phe Gly Thr Leu Leu Val Met Ala
            420                 425                 430

ttt cct tct tat cct gat ggc gtt gtt aga gca ttg tcg gtt ttc ggg   1344
Phe Pro Ser Tyr Pro Asp Gly Val Val Arg Ala Leu Ser Val Phe Gly
        435                 440                 445

tat gca ttt tgt tgt gca ccg ttt cta tgg gtc tcc ttt gtg aag atc   1392
Tyr Ala Phe Cys Cys Ala Pro Phe Leu Trp Val Ser Phe Val Lys Ile
    450                 455                 460

aca tcg cat ctt cag att atg att gac aaa gag gtt ttg ttt ccg cgg   1440
Thr Ser His Leu Gln Ile Met Ile Asp Lys Glu Val Leu Phe Pro Arg
465                 470                 475                 480

ttg ggt gaa tcc gga gtc act tct ggt ctc agc aaa ttg tac tga       1485
Leu Gly Glu Ser Gly Val Thr Ser Gly Leu Ser Lys Leu Tyr
                485                 490                 495
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Met Asp Leu Gln Arg Thr Leu Met Phe Ser Cys Trp Val Leu Ser Leu
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            20                  25                  30
Leu Glu Thr Glu Asn Ala Asn Ala Met Thr Ala Val Met Glu Arg Gly
        35                  40                  45
Leu Lys Thr Gln Arg Arg Pro Glu His Lys Asn Ala Tyr Ala Thr Met
    50                  55                  60
Met Tyr Met Gly Thr Pro Arg Asp Tyr Glu Phe Tyr Val Ala Thr Arg
65                  70                  75                  80
Val Leu Ile Arg Ser Leu Lys Ser Leu His Val Asp Ala Asp Ile Val
                85                  90                  95
Val Ile Ala Ser Leu Asp Val Pro Ile Asn Trp Ile His Ala Leu Glu
            100                 105                 110
Glu Glu Asp Gly Ala Lys Val Val Arg Val Glu Asn Leu Glu Asn Pro
        115                 120                 125
Tyr Lys Lys Gln Thr Asn Phe Asp Asn Arg Phe Lys Leu Ser Leu Asn
    130                 135                 140
Lys Leu Tyr Ala Trp Ser Leu Ser Asp Tyr Asp Arg Val Val Met Leu
145                 150                 155                 160
Asp Val Asp Asn Leu Phe Leu Lys Asn Thr Asp Glu Leu Phe Gln Cys
                165                 170                 175
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Gly Gln Phe Cys Ala Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly
            180                 185                 190
Leu Phe Val Leu Gln Pro Ser Met Glu Val Phe Arg Asp Met Leu His
        195                 200                 205
Glu Leu Glu Val Lys Arg Asp Asn Pro Asp Gly Ala Asp Gln Gly Phe
    210                 215                 220
Leu Val Ser Tyr Phe Ser Asp Leu Leu Asn Gln Pro Leu Phe Arg Pro
225                 230                 235                 240
Pro Pro Asp Asn Arg Thr Ala Leu Lys Gly His Phe Arg Leu Pro Leu
                245                 250                 255
Gly Tyr Gln Met Asp Ala Ser Tyr Tyr Tyr Leu Lys Leu Arg Trp Asn
            260                 265                 270
Val Pro Cys Gly Pro Asn Ser Val Ile Thr Phe Pro Gly Ala Val Trp
        275                 280                 285
Leu Lys Pro Trp Tyr Trp Trp Ser Trp Pro Val Leu Pro Leu Gly Leu
    290                 295                 300
Ser Trp His His Gln Arg Arg Tyr Thr Ile Ser Tyr Ser Ala Glu Met
305                 310                 315                 320
Pro Trp Val Leu Thr Gln Ala Val Phe Tyr Leu Gly Ile Ile Leu Val
                325                 330                 335
Thr Arg Leu Ala Arg Pro Asn Met Thr Lys Leu Cys Tyr Arg Arg Ser
            340                 345                 350
Asp Lys Asn Leu Ser Met Ile Gln Thr Ala Phe Lys Phe Val Ala Leu
        355                 360                 365
Leu Phe Ile Leu Ser Ala Tyr Ile Ile Pro Phe Phe Ile Ile Pro Gln
    370                 375                 380
Thr Ile His Pro Leu Ile Gly Trp Ser Leu Tyr Leu Thr Gly Ser Phe
385                 390                 395                 400
Ala Leu Ser Thr Ile Pro Ile Asn Ala Phe Leu Leu Pro Ile Leu Pro
                405                 410                 415
Val Ile Thr Pro Trp Leu Gly Ile Phe Gly Thr Leu Leu Val Met Ala
            420                 425                 430
Phe Pro Ser Tyr Pro Asp Gly Val Val Arg Ala Leu Ser Val Phe Gly
        435                 440                 445
Tyr Ala Phe Cys Cys Ala Pro Phe Leu Trp Val Ser Phe Val Lys Ile
    450                 455                 460
Thr Ser His Leu Gln Ile Met Ile Asp Lys Glu Val Leu Phe Pro Arg
465                 470                 475                 480
Leu Gly Glu Ser Gly Val Thr Ser Gly Leu Ser Lys Leu Tyr
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Met Asp Leu Gln Arg Gly Phe Val Phe Leu Ser Leu Val Leu Ser Phe
 1               5                  10                  15

atg ata atc gaa acg acg gcg tat cga gag aga cag ctg ctg ctg ctg   96
Met Ile Ile Glu Thr Thr Ala Tyr Arg Glu Arg Gln Leu Leu Leu Leu
            20                  25                  30

caa cca ccg caa gaa acg gcg ata gat acc gca aac gcg gtg gtg acg   144
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Gln Pro Pro Gln Glu Thr Ala Ile Asp Thr Ala Asn Ala Val Val Thr
 35                  40                  45

gtt caa gat cga ggt ttg aag acg cgg cga ccg gag cat aag aac gca   192
Val Gln Asp Arg Gly Leu Lys Thr Arg Arg Pro Glu His Lys Asn Ala
 50                  55                  60

tac gca acg atg atg tac atg ggg acg cca aga gac tac gag ttc tac   240
Tyr Ala Thr Met Met Tyr Met Gly Thr Pro Arg Asp Tyr Glu Phe Tyr
 65                  70                  75                  80

gtt gcg aca cgt gtt ttg atc aga tcg ttg aga agt ctt cac gtg gaa   288
Val Ala Thr Arg Val Leu Ile Arg Ser Leu Arg Ser Leu His Val Glu
                 85                  90                  95

gct gat ctc gtc gtc atc gct tct ctc gac gtt cct ctc cga tgg gtt   336
Ala Asp Leu Val Val Ile Ala Ser Leu Asp Val Pro Leu Arg Trp Val
            100                 105                 110

caa acc ttg gaa gag gaa gat gga gct aaa gtg gtg aga gtt gaa aat   384
Gln Thr Leu Glu Glu Glu Asp Gly Ala Lys Val Val Arg Val Glu Asn
        115                 120                 125

gtg gat aat cca tac agg aga cag acc aac ttc aac agt aga ttc aag   432
Val Asp Asn Pro Tyr Arg Arg Gln Thr Asn Phe Asn Ser Arg Phe Lys
    130                 135                 140

ctt act cta aac aag ctc tac gct tgg gct ttg tct gat tac gac cgt   480
Leu Thr Leu Asn Lys Leu Tyr Ala Trp Ala Leu Ser Asp Tyr Asp Arg
145                 150                 155                 160

gtg gtc atg cta gat gcc gat aac ctc ttt ctt aag aaa gcc gac gag   528
Val Val Met Leu Asp Ala Asp Asn Leu Phe Leu Lys Lys Ala Asp Glu
                165                 170                 175

ttg ttc cag tgt ggg cgc ttc tgt gcg gtc ttc att aac cct tgt atc   576
Leu Phe Gln Cys Gly Arg Phe Cys Ala Val Phe Ile Asn Pro Cys Ile
            180                 185                 190

ttc cac act ggt ctc ttc gtg ttg cag cca tca gtg gaa gtg ttc aag   624
Phe His Thr Gly Leu Phe Val Leu Gln Pro Ser Val Glu Val Phe Lys
        195                 200                 205

gac atg ctc cat gag cta caa gtt gga aga aag aat cct gat gga gct   672
Asp Met Leu His Glu Leu Gln Val Gly Arg Lys Asn Pro Asp Gly Ala
    210                 215                 220

gat caa ggt ttt ctt gtc agt tac ttc tct gat ctt ctt gac caa cct   720
Asp Gln Gly Phe Leu Val Ser Tyr Phe Ser Asp Leu Leu Asp Gln Pro
225                 230                 235                 240

ctc ttt agt cct ccg agt aac gga tct gta ctt aat ggt cac ttg aga   768
Leu Phe Ser Pro Pro Ser Asn Gly Ser Val Leu Asn Gly His Leu Arg
                245                 250                 255

ctt ccc tta ggc tac caa atg gac gct tct tat ttc tat ctt aag cta   816
Leu Pro Leu Gly Tyr Gln Met Asp Ala Ser Tyr Phe Tyr Leu Lys Leu
            260                 265                 270

aga tgg aac ata ccc tgt gga cca aac agt gtg att aca ttc ccg gga   864
Arg Trp Asn Ile Pro Cys Gly Pro Asn Ser Val Ile Thr Phe Pro Gly
        275                 280                 285
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gct gtt tgg tta aag cca tgg tac tgg tgg tca tgg cct gtt ctt cca 912
Ala Val Trp Leu Lys Pro Trp Tyr Trp Trp Ser Trp Pro Val Leu Pro
290 295 300

cta ggt ttc tca tgg cac gag cag cgt cgc gcc act ata ggg tac tca 960
Leu Gly Phe Ser Trp His Glu Gln Arg Arg Ala Thr Ile Gly Tyr Ser
305 310 315 320

gcc gaa atg cct ttg gtt ata atc caa gca atg ttt tac ctt gga atc 1008
Ala Glu Met Pro Leu Val Ile Ile Gln Ala Met Phe Tyr Leu Gly Ile
325 330 335

ata gtg gtt aca cga cta gct cgt cct aac ata acc aag cta tgt tat 1056
Ile Val Val Thr Arg Leu Ala Arg Pro Asn Ile Thr Lys Leu Cys Tyr
340 345 350

cgc cgc tct gac cgc aac tta aca acg atc caa gct ggt ttt aag ttg 1104
Arg Arg Ser Asp Arg Asn Leu Thr Thr Ile Gln Ala Gly Phe Lys Leu
355 360 365

atc gcg ctt ctc tct gta gtt gca gcc tac atc ttc cca ttc ttc acg 1152
Ile Ala Leu Leu Ser Val Val Ala Ala Tyr Ile Phe Pro Phe Phe Thr
370 375 380

atc cct cac act atc cac cca ctc atc ggc tgg tcg ctc tac ttg atg 1200
Ile Pro His Thr Ile His Pro Leu Ile Gly Trp Ser Leu Tyr Leu Met
385 390 395 400

gct tct ttt gct ctc tct tcc att tca atc aac act ctc ctc ctc cca 1248
Ala Ser Phe Ala Leu Ser Ser Ile Ser Ile Asn Thr Leu Leu Leu Pro
405 410 415

acg ctc cct gtt ctc act cca tgg cta gga att ctc ggc act ctc ctt 1296
Thr Leu Pro Val Leu Thr Pro Trp Leu Gly Ile Leu Gly Thr Leu Leu
420 425 430

gtc atg gcc ttc cct tgg tac cct gat gga gtg gtc aga gcc ttg tca 1344
Val Met Ala Phe Pro Trp Tyr Pro Asp Gly Val Val Arg Ala Leu Ser
435 440 445

gtt ttc gca tac gca ttt tgt tgc gca ccc ttt gtg tgg gtt tca ttc 1392
Val Phe Ala Tyr Ala Phe Cys Cys Ala Pro Phe Val Trp Val Ser Phe
450 455 460

cgc aaa atc aca tcg cac ctc cag gtt ttg att gag aaa gag gtg ttg 1440
Arg Lys Ile Thr Ser His Leu Gln Val Leu Ile Glu Lys Glu Val Leu
465 470 475 480

ttc ccg cga ttg gga gac tca ggg gtc act tca ggc ttc agc aaa ttg 1488
Phe Pro Arg Leu Gly Asp Ser Gly Val Thr Ser Gly Phe Ser Lys Leu
485 490 495

tat tag
Tyr 1494

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<212> PRT
<213> Arabidopsis thaliana

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Gln Pro Pro Gln Glu Thr Ala Ile Asp Thr Ala Asn Ala Val Val Thr
35 40 45
Val Gln Asp Arg Gly Leu Lys Thr Arg Arg Pro Glu His Lys Asn Ala
50 55 60
Tyr Ala Thr Met Met Tyr Met Gly Thr Pro Arg Asp Tyr Glu Phe Tyr
65 70 75 80
Val Ala Thr Arg Val Leu Ile Arg Ser Leu Arg Ser Leu His Val Glu
85 90 95
Ala Asp Leu Val Val Ile Ala Ser Leu Asp Val Pro Leu Arg Trp Val
100 105 110
Gln Thr Leu Glu Glu Glu Asp Gly Ala Lys Val Val Arg Val Glu Asn
115 120 125
Val Asp Asn Pro Tyr Arg Arg Gln Thr Asn Phe Asn Ser Arg Phe Lys
130 135 140
Leu Thr Leu Asn Lys Leu Tyr Ala Trp Ala Leu Ser Asp Tyr Asp Arg
145 150 155 160
Val Val Met Leu Asp Ala Asp Asn Leu Phe Leu Lys Lys Ala Asp Glu
165 170 175
Leu Phe Gln Cys Gly Arg Phe Cys Ala Val Phe Ile Asn Pro Cys Ile
180 185 190
Phe His Thr Gly Leu Phe Val Leu Gln Pro Ser Val Glu Val Phe Lys
195 200 205
Asp Met Leu His Glu Leu Gln Val Gly Arg Lys Asn Pro Asp Gly Ala
210 215 220
Asp Gln Gly Phe Leu Val Ser Tyr Phe Ser Asp Leu Leu Asp Gln Pro
225 230 235 240
Leu Phe Ser Pro Pro Ser Asn Gly Ser Val Leu Asn Gly His Leu Arg
245 250 255
Leu Pro Leu Gly Tyr Gln Met Asp Ala Ser Tyr Phe Tyr Leu Lys Leu
260 265 270
Arg Trp Asn Ile Pro Cys Gly Pro Asn Ser Val Ile Thr Phe Pro Gly
275 280 285
Ala Val Trp Leu Lys Pro Trp Tyr Trp Trp Ser Trp Pro Val Leu Pro
290 295 300
Leu Gly Phe Ser Trp His Glu Gln Arg Arg Ala Thr Ile Gly Tyr Ser
305 310 315 320
Ala Glu Met Pro Leu Val Ile Ile Gln Ala Met Phe Tyr Leu Gly Ile
325 330 335
Ile Val Val Thr Arg Leu Ala Arg Pro Asn Ile Thr Lys Leu Cys Tyr
340 345 350
Arg Arg Ser Asp Arg Asn Leu Thr Thr Ile Gln Ala Gly Phe Lys Leu
355 360 365
Ile Ala Leu Leu Ser Val Val Ala Ala Tyr Ile Phe Pro Phe Phe Thr
370 375 380
Ile Pro His Thr Ile His Pro Leu Ile Gly Trp Ser Leu Tyr Leu Met
385 390 395 400
Ala Ser Phe Ala Leu Ser Ser Ile Ser Ile Asn Thr Leu Leu Leu Pro
405 410 415
Thr Leu Pro Val Leu Thr Pro Trp Leu Gly Ile Leu Gly Thr Leu Leu
420 425 430
Val Met Ala Phe Pro Trp Tyr Pro Asp Gly Val Val Arg Ala Leu Ser
435 440 445
Val Phe Ala Tyr Ala Phe Cys Cys Ala Pro Phe Val Trp Val Ser Phe
450 455 460
Arg Lys Ile Thr Ser His Leu Gln Val Leu Ile Glu Lys Glu Val Leu
465 470 475 480
Phe Pro Arg Leu Gly Asp Ser Gly Val Thr Ser Gly Phe Ser Lys Leu

Tyr 485 490 495

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<213> Arabidopsis thaliana

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Met His Pro Ala Ser Cys Ser Leu Ser Leu Arg Lys Val Lys Leu Asn
 1               5                  10                  15

att ctt aca gtg aga atg aag ctc tct tct gaa agt ccc atg gcg ccg   96
Ile Leu Thr Val Arg Met Lys Leu Ser Ser Glu Ser Pro Met Ala Pro
            20                  25                  30

tca agt cag tca agt cat cga ctt tac att tcc agt gag aaa aca aaa  144
Ser Ser Gln Ser Ser His Arg Leu Tyr Ile Ser Ser Glu Lys Thr Lys
        35                  40                  45

acg aag aga ttc caa aga aac gga tac act ctc gat gtt gaa atg tgt  192
Thr Lys Arg Phe Gln Arg Asn Gly Tyr Thr Leu Asp Val Glu Met Cys
    50                  55                  60

gtc aac ttc tct agt ctg aaa ctt gtt ttg ttt ctt atg atg ctg gtt  240
Val Asn Phe Ser Ser Leu Lys Leu Val Leu Phe Leu Met Met Leu Val
65                  70                  75                  80

gct atg ttc aca ctc tac tgt tct cca ccg ttg caa att cct gaa gat  288
Ala Met Phe Thr Leu Tyr Cys Ser Pro Pro Leu Gln Ile Pro Glu Asp
                85                  90                  95

cca tca agt ttt gca aac aaa tgg ata cta gaa cct gct gta acc aca  336
Pro Ser Ser Phe Ala Asn Lys Trp Ile Leu Glu Pro Ala Val Thr Thr
            100                 105                 110

gat cct cgc tat ata gct aca tct gag atc aac tgg aac agt atg tca  384
Asp Pro Arg Tyr Ile Ala Thr Ser Glu Ile Asn Trp Asn Ser Met Ser
        115                 120                 125

ctt gtt gtt gag cat tac tta tct ggc aga agc gag tat caa gga att  432
Leu Val Val Glu His Tyr Leu Ser Gly Arg Ser Glu Tyr Gln Gly Ile
    130                 135                 140

ggc ttt cta aat ctc aac gat aac gag att aat cga tgg cag gtg gtc  480
Gly Phe Leu Asn Leu Asn Asp Asn Glu Ile Asn Arg Trp Gln Val Val
145                 150                 155                 160

ata aaa tct cac tgt cag cat ata gct ttg cat cta gac cat gct gca  528
Ile Lys Ser His Cys Gln His Ile Ala Leu His Leu Asp His Ala Ala
                165                 170                 175

agt aac ata act tgg aaa tct tta tac ccg gaa tgg att gac gag gaa  576
Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp Glu Glu
            180                 185                 190
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gaa aaa ttc aaa gtc ccc act tgt cct tct ctt cct tgg att caa gtt   624
Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile Gln Val
        195                 200                 205

cct gac aag tct cga atc gat ctt atc att gcc aag ctc cca tgt aac   672
Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro Cys Asn
    210                 215                 220

aag tca gga aaa tgg tca aga gat gtg gct aga ttg cac tta caa ctt   720
Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln Leu
225                 230                 235                 240

gca gca gct cga gtg gcg gca tct tct gaa ggg ctt cat gat gtt cat   768
Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp Val His
                245                 250                 255

gtg att ttg gta tca gat tgc ttt cca ata ccg aat ctt ttt acg ggt   816
Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly
            260                 265                 270

caa gaa ctt gtt gcc cgt caa gga aac ata tgg ctg tat aag cct aaa   864
Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro Lys
        275                 280                 285

ctt cac cag tta aga caa aag tta caa ctt cct gtt ggt tcc tgt gaa   912
Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys Glu
    290                 295                 300

ctt tct gtt cct ctt caa gct aaa gat aat ttc tac tcg gca aat gcc   960
Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Asn Ala
305                 310                 315                 320

aag aaa gaa gcg tac gcg acg atc ttg cac tca gat gat gct ttt gtc  1008
Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Asp Asp Ala Phe Val
                325                 330                 335

tgt gga gcc att gca gta gca cag agc att cga atg tca ggc tct act  1056
Cys Gly Ala Ile Ala Val Ala Gln Ser Ile Arg Met Ser Gly Ser Thr
            340                 345                 350

cgc aat ttg gta ata cta gtc gat gat tcg atc agt gaa tac cat aga  1104
Arg Asn Leu Val Ile Leu Val Asp Asp Ser Ile Ser Glu Tyr His Arg
        355                 360                 365

agt ggc ttg gaa tca gct gga tgg aag att cac aca ttt caa aga atc  1152
Ser Gly Leu Glu Ser Ala Gly Trp Lys Ile His Thr Phe Gln Arg Ile
    370                 375                 380

aga aac ccg aaa gct gaa gca aat gca tat aac caa tgg aac tac agc  1200
Arg Asn Pro Lys Ala Glu Ala Asn Ala Tyr Asn Gln Trp Asn Tyr Ser
385                 390                 395                 400

aaa ttc cgt ctt tgg gaa ttg aca gaa tac aac aag atc atc ttc att  1248
Lys Phe Arg Leu Trp Glu Leu Thr Glu Tyr Asn Lys Ile Ile Phe Ile
                405                 410                 415

gat gca gac atg ctt atc ctc aga aac atg gat ttc ctc ttc gag tac  1296
Asp Ala Asp Met Leu Ile Leu Arg Asn Met Asp Phe Leu Phe Glu Tyr
            420                 425                 430
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ccc gaa atc tcc aca act gga aac gac ggt acg ctc ttc aac tcc ggt   1344
Pro Glu Ile Ser Thr Thr Gly Asn Asp Gly Thr Leu Phe Asn Ser Gly
        435                 440                 445

cta atg gtg att gaa cca tca aat tca aca ttc cag tta cta atg gat   1392
Leu Met Val Ile Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met Asp
    450                 455                 460

cac atc aac gat atc aat tcc tac aat gga gga gac caa ggt tac ctt   1440
His Ile Asn Asp Ile Asn Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu
465                 470                 475                 480

aac gag ata ttc aca tgg tgg cat cgg att cca aaa cac atg aat ttc   1488
Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe
                485                 490                 495

ttg aag cat ttc tgg gaa gga gac aca cct aag cac agg aaa tct aag   1536
Leu Lys His Phe Trp Glu Gly Asp Thr Pro Lys His Arg Lys Ser Lys
            500                 505                 510

acg aga cta ttt gga gct gat cct ccg ata ctc tac gtt ctt cat tac   1584
Thr Arg Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His Tyr
        515                 520                 525

cta ggt tac aac aaa cca tgg gta tgc ttc aga gac tac gat tgc aat   1632
Leu Gly Tyr Asn Lys Pro Trp Val Cys Phe Arg Asp Tyr Asp Cys Asn
    530                 535                 540

tgg aat gtc gtt gga tac cat caa ttc gcg agc gat gaa gca cac aaa   1680
Trp Asn Val Val Gly Tyr His Gln Phe Ala Ser Asp Glu Ala His Lys
545                 550                 555                 560

act tgg tgg aga gtg cac gac gcg atg cct aag aaa ttg cag agg ttt   1728
Thr Trp Trp Arg Val His Asp Ala Met Pro Lys Lys Leu Gln Arg Phe
                565                 570                 575

tgt cta ctg agt tcg aaa caa aag gcg caa ctt gag tgg gat cgg aga   1776
Cys Leu Leu Ser Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg
            580                 585                 590

caa gct gag aaa gcg aat tac aga gac gga cat tgg agg att aag atc   1824
Gln Ala Glu Lys Ala Asn Tyr Arg Asp Gly His Trp Arg Ile Lys Ile
        595                 600                 605

aaa gat aag aga ctt acg act tgt ttt gaa gat ttc tgt ttc tgg gag   1872
Lys Asp Lys Arg Leu Thr Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu
    610                 615                 620

agt atg ctt tgg cat tgg ggt gag act cag acc aac tcc acc gtc gct   1920
Ser Met Leu Trp His Trp Gly Glu Thr Gln Thr Asn Ser Thr Val Ala
625                 630                 635                 640

gct gat tct tcc tcc acc gcg taa                                  1944
Ala Asp Ser Ser Ser Thr Ala
                645
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Met His Pro Ala Ser Cys Ser Leu Ser Leu Arg Lys Val Lys Leu Asn
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Ser Ser Gln Ser Ser His Arg Leu Tyr Ile Ser Ser Glu Lys Thr Lys
35 40 45
Thr Lys Arg Phe Gln Arg Asn Gly Tyr Thr Leu Asp Val Glu Met Cys
50 55 60
Val Asn Phe Ser Ser Leu Lys Leu Val Leu Phe Leu Met Met Leu Val
65 70 75 80
Ala Met Phe Thr Leu Tyr Cys Ser Pro Pro Leu Gln Ile Pro Glu Asp
85 90 95
Pro Ser Ser Phe Ala Asn Lys Trp Ile Leu Glu Pro Ala Val Thr Thr
100 105 110
Asp Pro Arg Tyr Ile Ala Thr Ser Glu Ile Asn Trp Asn Ser Met Ser
115 120 125
Leu Val Val Glu His Tyr Leu Ser Gly Arg Ser Glu Tyr Gln Gly Ile
130 135 140
Gly Phe Leu Asn Leu Asn Asp Asn Glu Ile Asn Arg Trp Gln Val Val
145 150 155 160
Ile Lys Ser His Cys Gln His Ile Ala Leu His Leu Asp His Ala Ala
165 170 175
Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp Glu Glu
180 185 190
Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile Gln Val
195 200 205
Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro Cys Asn
210 215 220
Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln Leu
225 230 235 240
Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp Val His
245 250 255
Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly
260 265 270
Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro Lys
275 280 285
Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys Glu
290 295 300
Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Asn Ala
305 310 315 320
Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Asp Asp Ala Phe Val
325 330 335
Cys Gly Ala Ile Ala Val Ala Gln Ser Ile Arg Met Ser Gly Ser Thr
340 345 350
Arg Asn Leu Val Ile Leu Val Asp Asp Ser Ile Ser Glu Tyr His Arg
355 360 365
Ser Gly Leu Glu Ser Ala Gly Trp Lys Ile His Thr Phe Gln Arg Ile
370 375 380
Arg Asn Pro Lys Ala Glu Ala Asn Ala Tyr Asn Gln Trp Asn Tyr Ser
385 390 395 400
Lys Phe Arg Leu Trp Glu Leu Thr Glu Tyr Asn Lys Ile Ile Phe Ile
405 410 415
Asp Ala Asp Met Leu Ile Leu Arg Asn Met Asp Phe Leu Phe Glu Tyr
420 425 430
Pro Glu Ile Ser Thr Thr Gly Asn Asp Gly Thr Leu Phe Asn Ser Gly
435 440 445
Leu Met Val Ile Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met Asp
450 455 460
His Ile Asn Asp Ile Asn Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu
465 470 475 480
Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe

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                485                 490                 495
Leu Lys His Phe Trp Glu Gly Asp Thr Pro Lys His Arg Lys Ser Lys
            500                 505                 510
Thr Arg Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His Tyr
        515                 520                 525
Leu Gly Tyr Asn Lys Pro Trp Val Cys Phe Arg Asp Tyr Asp Cys Asn
    530                 535                 540
Trp Asn Val Val Gly Tyr His Gln Phe Ala Ser Asp Glu Ala His Lys
545                 550                 555                 560
Thr Trp Trp Arg Val His Asp Ala Met Pro Lys Lys Leu Gln Arg Phe
                565                 570                 575
Cys Leu Leu Ser Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg
            580                 585                 590
Gln Ala Glu Lys Ala Asn Tyr Arg Asp Gly His Trp Arg Ile Lys Ile
        595                 600                 605
Lys Asp Lys Arg Leu Thr Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu
    610                 615                 620
Ser Met Leu Trp His Trp Gly Glu Thr Gln Thr Asn Ser Thr Val Ala
625                 630                 635                 640
Ala Asp Ser Ser Ser Thr Ala
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  1               5                  10                  15

tca aaa gag aag aca agt agg agg aga ttt caa aga att gag aag ggt   96
Ser Lys Glu Lys Thr Ser Arg Arg Arg Phe Gln Arg Ile Glu Lys Gly
             20                  25                  30

gtc aag ttc aac act ctg aaa ctt gtg ttg att tgt ata atg ctt gga   144
Val Lys Phe Asn Thr Leu Lys Leu Val Leu Ile Cys Ile Met Leu Gly
         35                  40                  45

gct ttg ttc acg atc tac cgt ttt cgt tat cca ccg cta caa att cct   192
Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro
     50                  55                  60

gaa att cca act agt ttt ggt ctt act act gat cct cgc tat gta gct   240
Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala
 65                  70                  75                  80

aca gct gag atc aac tgg aac cat atg tca aat ctt gtt gag aag cac   288
Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His
                 85                  90                  95

gta ttt ggt aga agc gag tat caa gga att ggt ctt ata aat ctt aac   336
Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn
            100                 105                 110
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gat aac gag att gat cga ttc aag gag gta acg aaa tct gac tgt gat   384
Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp
        115                 120                 125

cat gta gct ttg cat cta gat tat gct gca aag aac ata aca tgg gaa   432
His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu
    130                 135                 140

tct tta tac ccg gaa tgg att gat gaa gtt gaa gaa ttc gaa gtc cct   480
Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro
145                 150                 155                 160

act tgt cct tct ctg cct ttg att caa att cct ggc aag cct cgg att   528
Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile
                165                 170                 175

gat ctt gta att gcc aag ctt ccg tgt gat aaa tca gga aaa tgg tct   576
Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser
            180                 185                 190

aga gat gtg gct cgc ttg cat tta caa ctt gca gca gct cga gtg gcg   624
Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Ala Arg Val Ala
        195                 200                 205

gct tct tct aaa gga ctt cat aat gtt cat gtg att ttg gta tct gat   672
Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp
    210                 215                 220

tgc ttt cca ata ccg aat ctt ttt acg ggt caa gaa ctt gtt gcc cgt   720
Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg
225                 230                 235                 240

caa gga aac ata tgg ctg tat aag cct aat ctt cac cag cta aga caa   768
Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln
                245                 250                 255

aag tta cag ctt cct gtt ggt tcc tgt gaa ctt tct gtt cct ctt caa   816
Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln
            260                 265                 270

gct aaa gat aat ttc tac tcc gca ggt gca aag aaa gaa gct tac gcg   864
Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala
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act atc ttg cat tct gcc caa ttt tat gtc tgt gga gcc att gca gct   912
Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala
    290                 295                 300

gca cag agc att cga atg tca ggc tct act cgt gat ctg gtc ata ctt   960
Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu
305                 310                 315                 320

gtt gat gaa acg ata agc gaa tac cat aaa agt ggc ttg gta gct gct  1008
Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala
                325                 330                 335

gga tgg aag att caa atg ttt caa aga atc agg aac ccg aat gct gta  1056
Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val
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cca aat gcc tac aac gaa tgg aac tac agc aag ttt cgt ctt tgg caa  1104
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Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
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Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile
370                 375                 380

ctg aga aac att gat ttc ctc ttc gag ttc cct gag ata tca gca act   1200
Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr
385                 390                 395                 400

gga aac aat gct acg ctc ttc aac tct ggt cta atg gtg gtt gag cca   1248
Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro
                405                 410                 415

tct aat tca aca ttc cag tta cta atg gat aac att aat gaa gtt gtg   1296
Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val
            420                 425                 430

tct tac aac gga gga gac caa ggt tac ctt aac gag ata ttc aca tgg   1344
Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
        435                 440                 445

tgg cat cgg att cca aaa cac atg aat ttc ttg aag cat ttc tgg gaa   1392
Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu
    450                 455                 460

gga gac gaa cct gag att aaa aaa atg aag acg agt cta ttt gga gct   1440
Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala
465                 470                 475                 480

gat cct ccg atc cta tac gtt ctt cat tac cta ggt tat aac aaa ccc   1488
Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
                485                 490                 495

tgg tta tgc ttc aga gac tat gac tgc aat tgg aat gtc gat att ttc   1536
Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
            500                 505                 510

cag gaa ttt gct agt gac gag gct cat aaa acc tgg tgg aga gtg cac   1584
Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
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gac gca atg cct gaa aac ttg cat aag ttc tgt cta cta aga tcg aaa   1632
Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
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cag aag gcg caa ctt gaa tgg gat agg aga caa gca gag aaa ggg aac   1680
Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
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tac aaa gat gga cat tgg aag ata aag atc aaa gac aag aga ctt aag   1728
Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys
                565                 570                 575

act tgt ttc gaa gat ttc tgc ttt tgg gag agt atg ctt tgg cat tgg   1776
Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp
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ggt gag acg aac tct acc aac aat tct tcc acc acc acc act tca tca   1824
Gly Glu Thr Asn Ser Thr Asn Asn Ser Ser Thr Thr Thr Thr Ser Ser
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Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro
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Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala
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Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His
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Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn
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Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp
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His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu
130 135 140
Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro
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Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile
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Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser
180 185 190
Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Ala Arg Val Ala
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Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp
210 215 220
Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg
225 230 235 240
Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln
245 250 255
Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln
260 265 270
Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala
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Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala
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Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu
305 310 315 320
Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala
325 330 335
Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val
340 345 350

Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
355 360 365
Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile
370 375 380

Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr
385 390 395 400
Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro
405 410 415
Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val
420 425 430
Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
435 440 445
Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu
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Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala
465 470 475 480
Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
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Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
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Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
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Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
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Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
545 550 555 560
Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys
565 570 575
Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp
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Asp Ser Arg Arg Arg Leu Ser Ala Ser Ile Glu Ala Ile Cys Lys Arg
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aga ttc cgg aga aat agc aaa gga ggt ggc aga tcg gat atg gtg aaa 144
Arg Phe Arg Arg Asn Ser Lys Gly Gly Gly Arg Ser Asp Met Val Lys
35 40 45

ccg ttt aat atc ata aat ttt tcg aca caa gac aaa aac agt agt tgt 192
Pro Phe Asn Ile Ile Asn Phe Ser Thr Gln Asp Lys Asn Ser Ser Cys
50 55 60

tgt tgt ttc acc aag ttt cag atc gtg aag ctt ctc ttg ttt atc ctt 240
Cys Cys Phe Thr Lys Phe Gln Ile Val Lys Leu Leu Leu Phe Ile Leu
65 70 75 80

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ctc tct gcc act ctc ttc acc att atc tat tct cct gaa gct tat cat   288
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                85                  90                  95

cat tct ctt tcc cac tca tct tct cga tgg ata tgg aga aga caa gat   336
His Ser Leu Ser His Ser Ser Ser Arg Trp Ile Trp Arg Arg Gln Asp
            100                 105                 110

cca cgt tac ttc tcg gat ctg gat ata aac tgg gac gat gtg act aaa   384
Pro Arg Tyr Phe Ser Asp Leu Asp Ile Asn Trp Asp Asp Val Thr Lys
        115                 120                 125

acc ctt gag aac atc gaa gaa ggc cgt acg atc ggt gtc ttg aat ttt   432
Thr Leu Glu Asn Ile Glu Glu Gly Arg Thr Ile Gly Val Leu Asn Phe
    130                 135                 140

gat tcg aac gag atc caa cga tgg aga gaa gta tcc aag agc aag gac   480
Asp Ser Asn Glu Ile Gln Arg Trp Arg Glu Val Ser Lys Ser Lys Asp
145                 150                 155                 160

aat ggg gat gaa gaa aaa gtt gtt gta ttg aat cta gat tac gca gac   528
Asn Gly Asp Glu Glu Lys Val Val Val Leu Asn Leu Asp Tyr Ala Asp
                165                 170                 175

aag aat gtg act tgg gac gca cta tat cca gag tgg atc gat gag gag   576
Lys Asn Val Thr Trp Asp Ala Leu Tyr Pro Glu Trp Ile Asp Glu Glu
            180                 185                 190

caa gaa aca gag gtc cct gtt tgt cct aat atc ccg aac att aag gta   624
Gln Glu Thr Glu Val Pro Val Cys Pro Asn Ile Pro Asn Ile Lys Val
        195                 200                 205

cct aca aga aga ctc gat ctg atc gtc gtg aaa ctt cct tgt cgg aaa   672
Pro Thr Arg Arg Leu Asp Leu Ile Val Val Lys Leu Pro Cys Arg Lys
    210                 215                 220

gaa ggg aat tgg tcg aga gac gtc ggg aga ttg cat cta cag cta gcg   720
Glu Gly Asn Trp Ser Arg Asp Val Gly Arg Leu His Leu Gln Leu Ala
225                 230                 235                 240

gct gca act gtg gcg gct tcg gcc aaa ggg ttt ttc agg gga cat gtg   768
Ala Ala Thr Val Ala Ala Ser Ala Lys Gly Phe Phe Arg Gly His Val
                245                 250                 255

ttt ttt gta tct aga tgc ttt ccg att ccg aat ctt ttc cgg tgt aaa   816
Phe Phe Val Ser Arg Cys Phe Pro Ile Pro Asn Leu Phe Arg Cys Lys
            260                 265                 270

gat ctt gtg tct cgg aga ggc gat gtt tgg ttg tac aaa cct aat ctt   864
Asp Leu Val Ser Arg Arg Gly Asp Val Trp Leu Tyr Lys Pro Asn Leu
        275                 280                 285

gat acc ttg aga gac aag ctt cag ctg cct gta ggg tct tgt gag cta   912
Asp Thr Leu Arg Asp Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu
    290                 295                 300

tct ctt cct ctt ggc atc caa gat agg cca agc tta gga aac cct aaa   960
Ser Leu Pro Leu Gly Ile Gln Asp Arg Pro Ser Leu Gly Asn Pro Lys
305                 310                 315                 320

aga gaa gct tac gca aca att ctt cac tca gct cac gtt tac gtc tgc  1008
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Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala His Val Tyr Val Cys
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ggt gca atc gcc gcg gct cag agc ata aga cag tct ggt tcg acg aga   1056
Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Gln Ser Gly Ser Thr Arg
            340                 345                 350

gac ctt gtt atc ctt gtt gat gac aac atc agc ggt tac cac cgg agt   1104
Asp Leu Val Ile Leu Val Asp Asp Asn Ile Ser Gly Tyr His Arg Ser
        355                 360                 365

gga cta gaa gcc gcg ggt tgg caa atc cgg acg ata cag agg att cga   1152
Gly Leu Glu Ala Ala Gly Trp Gln Ile Arg Thr Ile Gln Arg Ile Arg
    370                 375                 380

aac cct aag gca gag aaa gat gct tac aac gaa tgg aac tac agc aag   1200
Asn Pro Lys Ala Glu Lys Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys
385                 390                 395                 400

ttc cgg cta tgg cag ctg act gat tac gac aaa atc att ttc atc gac   1248
Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp
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gcg gat ctc tta atc ttg aga aac atc gat ttc ttg ttc tcg atg cct   1296
Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Ser Met Pro
            420                 425                 430

gag atc tca gct aca gga aac aat gga act ctg ttt aat tca gga gtt   1344
Glu Ile Ser Ala Thr Gly Asn Asn Gly Thr Leu Phe Asn Ser Gly Val
        435                 440                 445

atg gtg atc gag cct tgc aac tgt acg ttt cag ctt ctg atg gaa cat   1392
Met Val Ile Glu Pro Cys Asn Cys Thr Phe Gln Leu Leu Met Glu His
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ata aac gag att gag tct tat aac ggt gga gat caa ggt tac tta aac   1440
Ile Asn Glu Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn
465                 470                 475                 480

gag gta ttc aca tgg tgg cac cgg att cca aaa cat atg aat ttc ttg   1488
Glu Val Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe Leu
                485                 490                 495

aag cat ttt tgg att ggc gat gaa gat gac gcg aaa cgc aag aaa aca   1536
Lys His Phe Trp Ile Gly Asp Glu Asp Asp Ala Lys Arg Lys Lys Thr
            500                 505                 510

gag ctt ttt gga gca gag cct cct gtt ctt tat gtt ctt cat tac ctt   1584
Glu Leu Phe Gly Ala Glu Pro Pro Val Leu Tyr Val Leu His Tyr Leu
        515                 520                 525

ggg atg aag ccg tgg tta tgt tac cgt gac tac gac tgt aac ttc aac   1632
Gly Met Lys Pro Trp Leu Cys Tyr Arg Asp Tyr Asp Cys Asn Phe Asn
    530                 535                 540

tcc gac ata ttc gtt gag ttt gct acc gat atc gct cat cga aaa tgg   1680
Ser Asp Ile Phe Val Glu Phe Ala Thr Asp Ile Ala His Arg Lys Trp
545                 550                 555                 560

tgg atg gtc cac gac gcc atg cca cag gaa ctt cac caa ttc tgt tac   1728
Trp Met Val His Asp Ala Met Pro Gln Glu Leu His Gln Phe Cys Tyr
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gag gcc gca aat tat gcc gac ggt cat tgg aaa ata aga gta aag gac   1824
Glu Ala Ala Asn Tyr Ala Asp Gly His Trp Lys Ile Arg Val Lys Asp
            595                 600                 605

ccg aga ttc aaa att tgc atc gac aaa tta tgt aat tgg aaa agt atg   1872
Pro Arg Phe Lys Ile Cys Ile Asp Lys Leu Cys Asn Trp Lys Ser Met
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ctg cgg cat tgg ggc gaa tca aat tgg act gac tac gag tct ttt gtt   1920
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ccc acc cca cca gcc att acc gta gac cgg aga tca tca ctt ccc ggc   1968
Pro Thr Pro Pro Ala Ile Thr Val Asp Arg Arg Ser Ser Leu Pro Gly
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cat aac ttg tga cgcaataatt atacatactt attaatggat ttcatgagtt       2020
His Asn Leu
            660

ttttggtttg aattgttgct gcgagattag gtgaatatca gttgtgtaac tatatctttt 2080

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Pro Phe Asn Ile Ile Asn Phe Ser Thr Gln Asp Lys Asn Ser Ser Cys
    50                  55                  60
Cys Cys Phe Thr Lys Phe Gln Ile Val Lys Leu Leu Leu Phe Ile Leu
65                  70                  75                  80
Leu Ser Ala Thr Leu Phe Thr Ile Ile Tyr Ser Pro Glu Ala Tyr His
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His Ser Leu Ser His Ser Ser Ser Arg Trp Ile Trp Arg Arg Gln Asp
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Pro Arg Tyr Phe Ser Asp Leu Asp Ile Asn Trp Asp Asp Val Thr Lys
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Asp Ser Asn Glu Ile Gln Arg Trp Arg Glu Val Ser Lys Ser Lys Asp
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Asn Gly Asp Glu Glu Lys Val Val Val Leu Asn Leu Asp Tyr Ala Asp
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Lys Asn Val Thr Trp Asp Ala Leu Tyr Pro Glu Trp Ile Asp Glu Glu
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Gln Glu Thr Glu Val Pro Val Cys Pro Asn Ile Pro Asn Ile Lys Val
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275 280 285
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290 295 300
Ser Leu Pro Leu Gly Ile Gln Asp Arg Pro Ser Leu Gly Asn Pro Lys
305 310 315 320
Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala His Val Tyr Val Cys
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Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Gln Ser Gly Ser Thr Arg
340 345 350
Asp Leu Val Ile Leu Val Asp Asp Asn Ile Ser Gly Tyr His Arg Ser
355 360 365
Gly Leu Glu Ala Ala Gly Trp Gln Ile Arg Thr Ile Gln Arg Ile Arg
370 375 380
Asn Pro Lys Ala Glu Lys Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys
385 390 395 400
Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp
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Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Ser Met Pro
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Glu Ile Ser Ala Thr Gly Asn Asn Gly Thr Leu Phe Asn Ser Gly Val
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Met Val Ile Glu Pro Cys Asn Cys Thr Phe Gln Leu Leu Met Glu His
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Ile Asn Glu Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn
465 470 475 480
Glu Val Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe Leu
485 490 495
Lys His Phe Trp Ile Gly Asp Glu Asp Asp Ala Lys Arg Lys Lys Thr
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Glu Leu Phe Gly Ala Glu Pro Pro Val Leu Tyr Val Leu His Tyr Leu
515 520 525
Gly Met Lys Pro Trp Leu Cys Tyr Arg Asp Tyr Asp Cys Asn Phe Asn
530 535 540
Ser Asp Ile Phe Val Glu Phe Ala Thr Asp Ile Ala His Arg Lys Trp
545 550 555 560
Trp Met Val His Asp Ala Met Pro Gln Glu Leu His Gln Phe Cys Tyr
565 570 575
Leu Arg Ser Lys Gln Lys Ala Gln Leu Glu Tyr Asp Arg Arg Gln Ala
580 585 590
Glu Ala Ala Asn Tyr Ala Asp Gly His Trp Lys Ile Arg Val Lys Asp
595 600 605
Pro Arg Phe Lys Ile Cys Ile Asp Lys Leu Cys Asn Trp Lys Ser Met
610 615 620
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Figure 1

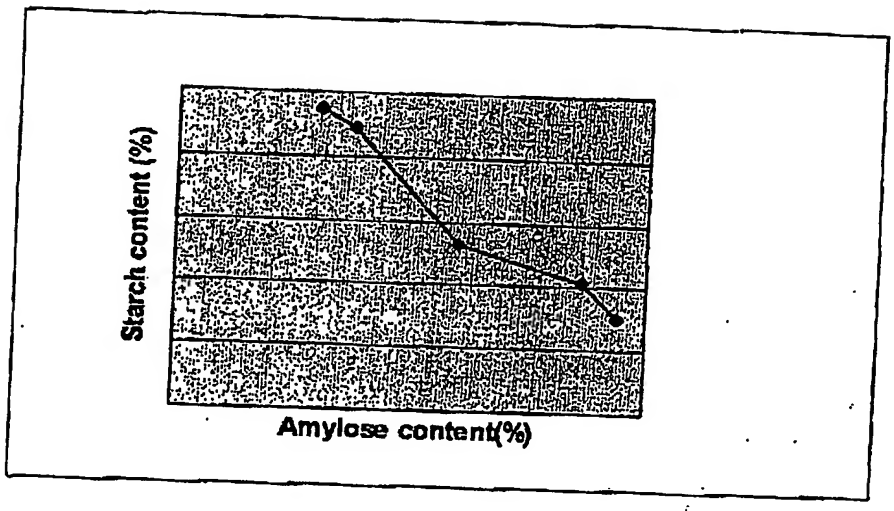
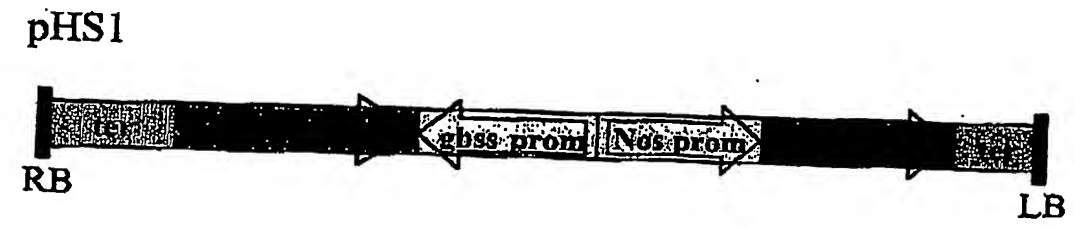


Figure 2

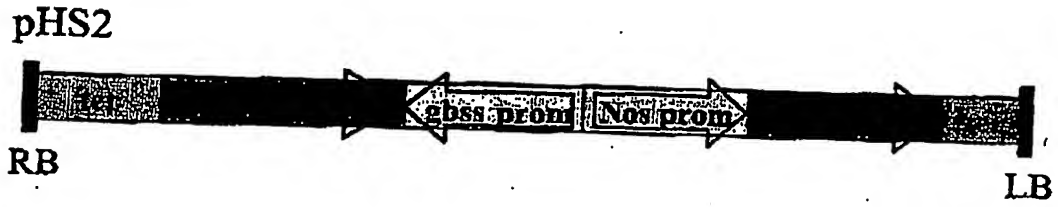
Variety/line	Mother variety	Dm %	Fructose % of Dm	Glucose % of Dm	Sucrose % of Dm
Producent		27,8	1,8	2,2	3,1
Prevalent		27,1	1,2	1,7	2,7
Dinamo		27,7	0,8	1,7	2,2
Kuras		27,9	1,8	2,2	2,4
AM98-2012	Producent	22,3	3,1	4,0	4,7
AM98-2019	Prevalent	18,4	2,9	4,4	3,8
AM98-2021	Prevalent	17,1	3,1	5,9	3,5
AM99-2002	Dinamo	19,3	2,4	3,8	3,0
AM99-2003	Dinamo	18,8	2,5	5,5	3,2
AM99-2004	Dinamo	11,7	4,0	6,8	2,1
AM00-2040	Kuras	21,0	5,0	6,3	3,5
AM00-2041	Kuras	19,3	5,6	7,1	3,0

Figure 3

3 a) pHS1 for gene-inhibition of StGH1



3 b) pHS2 for gene-inhibition of StGH2



3 c) pHS3 for over-expression of StGH1

3 d) pHS4 for over-expression of StGH2

Figure 4

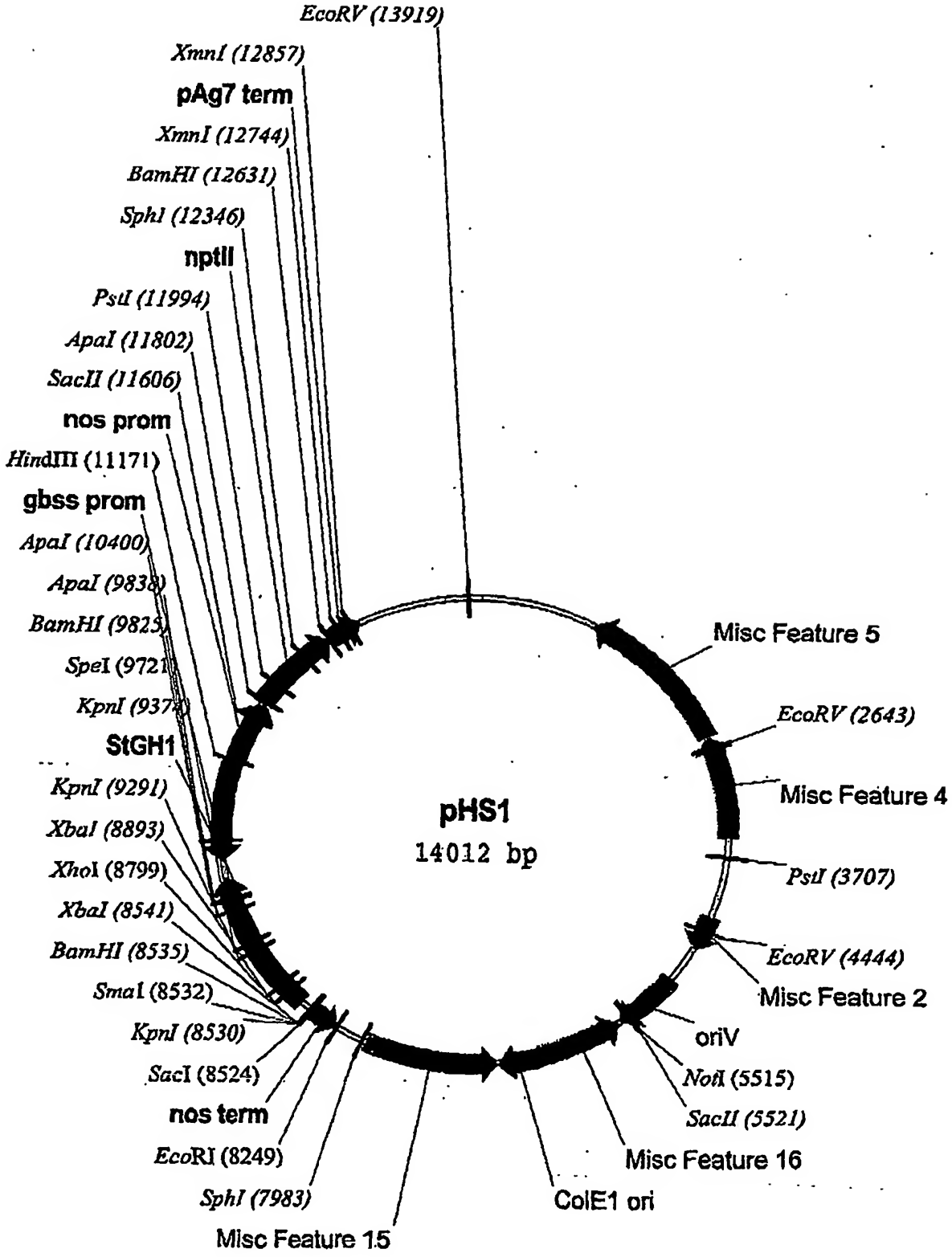

EcoRV (13919)
XmnI (12857)
pAg7 term
XmnI (12744)
BamHI (12631)
SphI (12346)
nptII
PstI (11994)
ApaI (11802)
SacII (11606)
nos prom
HindIII (11171)
gbss prom
ApaI (10400)
ApaI (9838)
BamHI (9825)
SpeI (9721)
KpnI (9379)
StGH1
KpnI (9291)
XbaI (8893)
XhoI (8799)
XbaI (8541)
BamHI (8535)
SmaI (8532)
KpnI (8530)
SacI (8524)
nos term
EcoRI (8249)
SphI (7983)
Misc Feature 15
ColE1 ori
Misc Feature 16
SacII (5521)
NotI (5515)
oriV
Misc Feature 2
EcoRV (4444)
PstI (3707)
Misc Feature 4
EcoRV (2643)
Misc Feature 5
pHS1
14012 bp

Figure 5

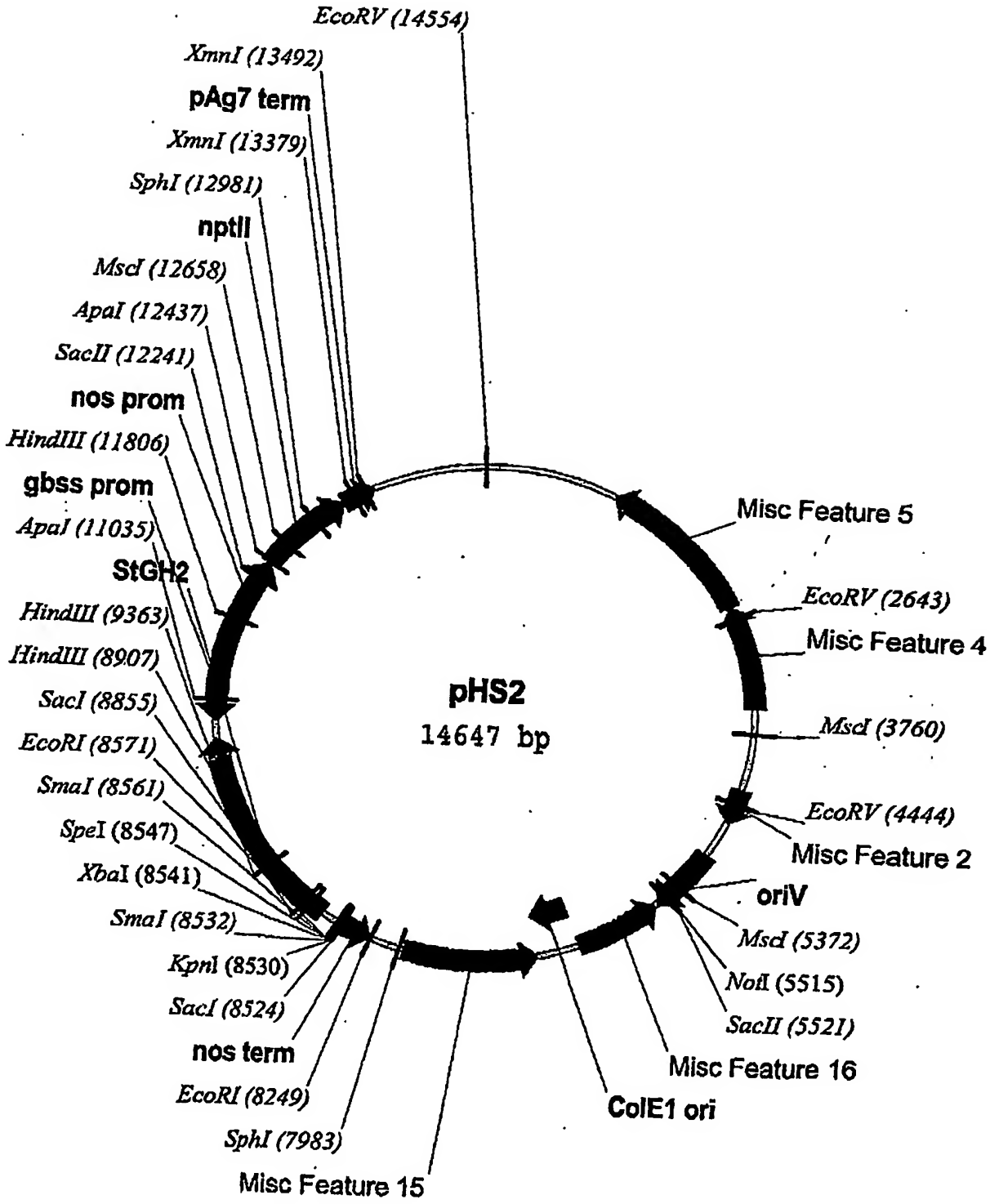

EcoRV (14554)
XmnI (13492)
pAg7 term
XmnI (13379)
SphI (12981)
nptII
MscI (12658)
ApaI (12437)
SacII (12241)
nos prom
HindIII (11806)
gbss prom
ApaI (11035)
StGH2
HindIII (9363)
HindIII (8907)
SacI (8855)
EcoRI (8571)
SmaI (8561)
SpeI (8547)
XbaI (8541)
SmaI (8532)
KpnI (8530)
SacI (8524)
nos term
EcoRI (8249)
SphI (7983)
Misc Feature 15
ColE1 ori
Misc Feature 16
SacII (5521)
NotI (5515)
MscI (5372)
oriV
Misc Feature 2
EcoRV (4444)
MscI (3760)
Misc Feature 4
EcoRV (2643)
Misc Feature 5
pHS2
14647 bp

Figure 6

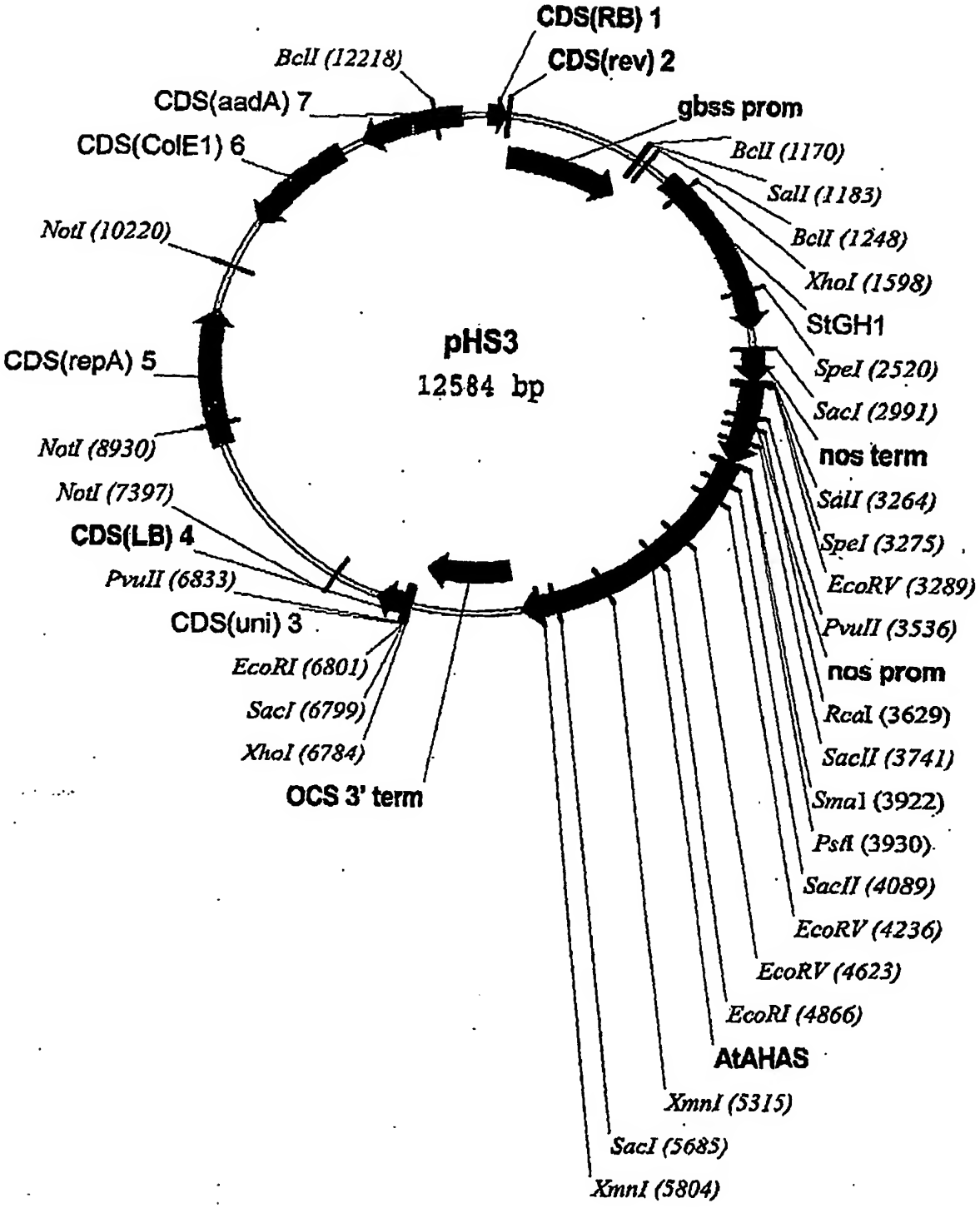

pHS3
12584 bp
CDS(RB) 1
CDS(rev) 2
gbss prom
BclI (1170)
SalI (1183)
BclI (1248)
XhoI (1598)
StGH1
SpeI (2520)
SacI (2991)
nos term
SalI (3264)
SpeI (3275)
EcoRV (3289)
PvuII (3536)
nos prom
RcaI (3629)
SacII (3741)
SmaI (3922)
PstI (3930)
SacII (4089)
EcoRV (4236)
EcoRV (4623)
EcoRI (4866)
AtAHAS
XmnI (5315)
SacI (5685)
XmnI (5804)
OCS 3' term
XhoI (6784)
SacI (6799)
EcoRI (6801)
CDS(uni) 3
PvuII (6833)
CDS(LB) 4
NotI (7397)
NotI (8930)
CDS(repA) 5
NotI (10220)
CDS(ColE1) 6
CDS(aadA) 7
BclI (12218)

Figure 7

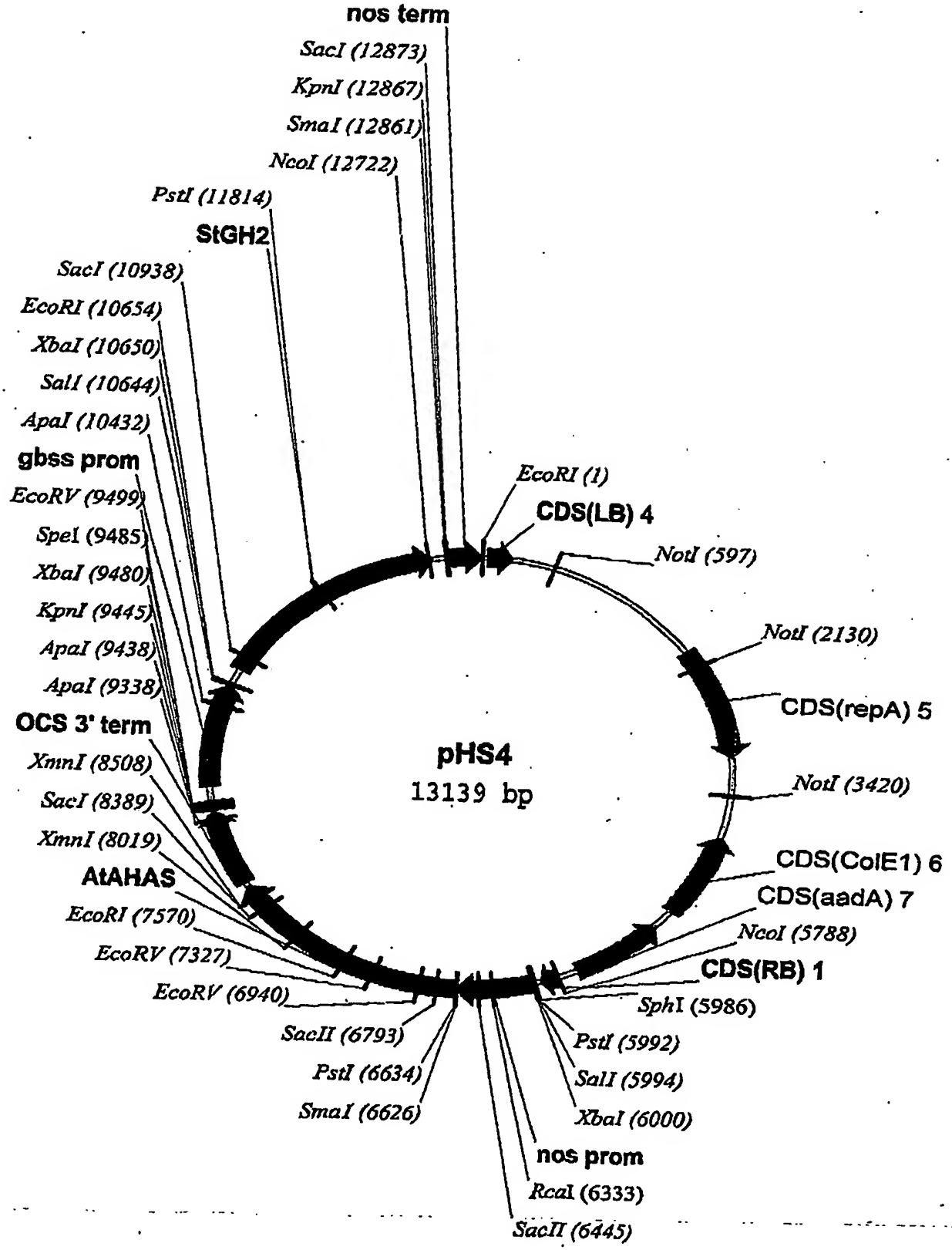

nos term
SacI (12873)
KpnI (12867)
SmaI (12861)
NcoI (12722)
PstI (11814)
StGH2
SacI (10938)
EcoRI (10654)
XbaI (10650)
SalI (10644)
ApaI (10432)
gbss prom
EcoRV (9499)
SpeI (9485)
XbaI (9480)
KpnI (9445)
ApaI (9438)
ApaI (9338)
OCS 3' term
XmnI (8508)
SacI (8389)
XmnI (8019)
AtAHAS
EcoRI (7570)
EcoRV (7327)
EcoRV (6940)
SacII (6793)
PstI (6634)
SmaI (6626)
nos prom
RcaI (6333)
SacII (6445)
XbaI (6000)
SalI (5994)
PstI (5992)
SphI (5986)
CDS(RB) 1
NcoI (5788)
CDS(aadA) 7
CDS(ColE1) 6
NotI (3420)
CDS(repA) 5
NotI (2130)
NotI (597)
CDS(LB) 4
EcoRI (1)
pHS4
13139 bp

Figure 8

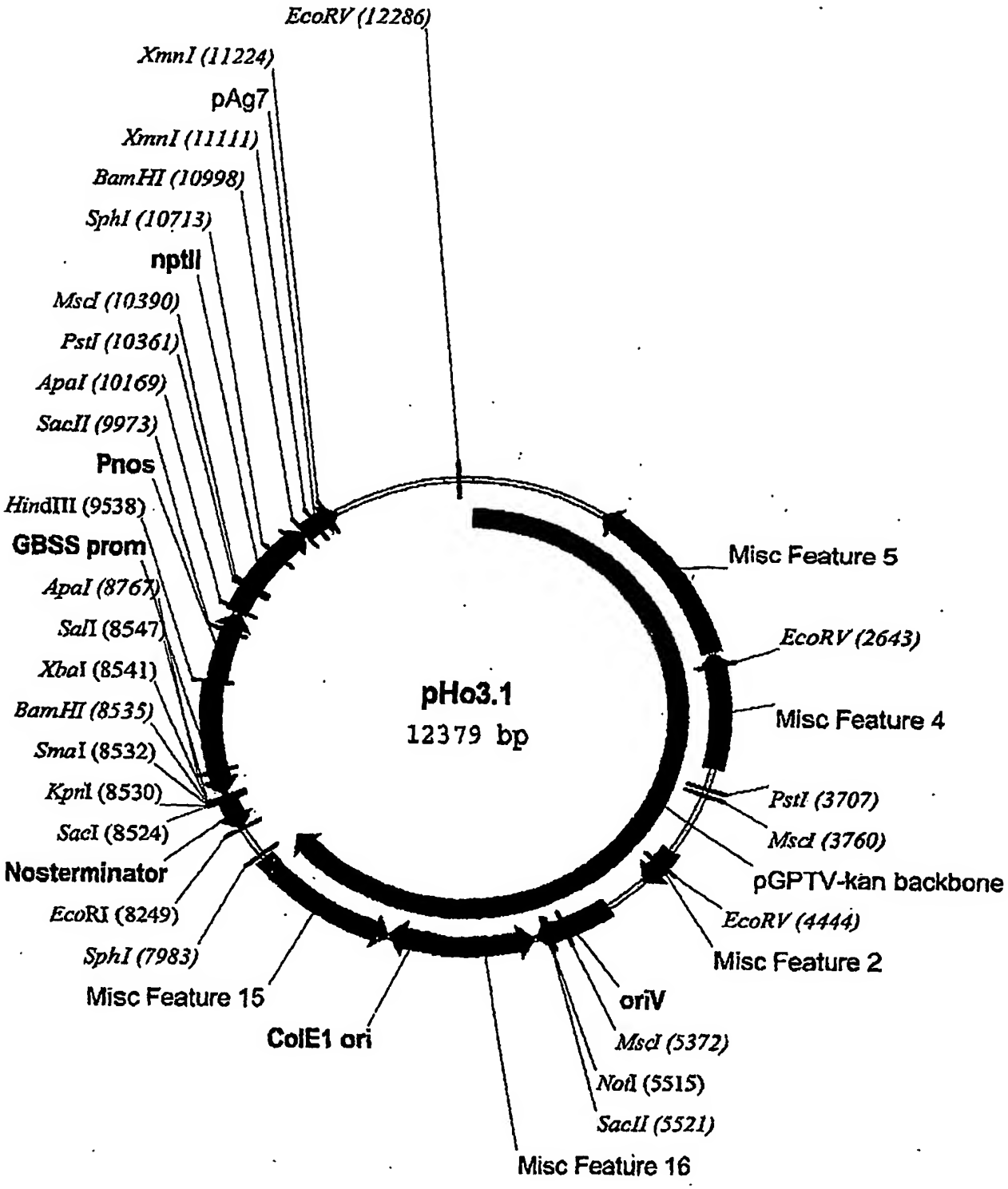
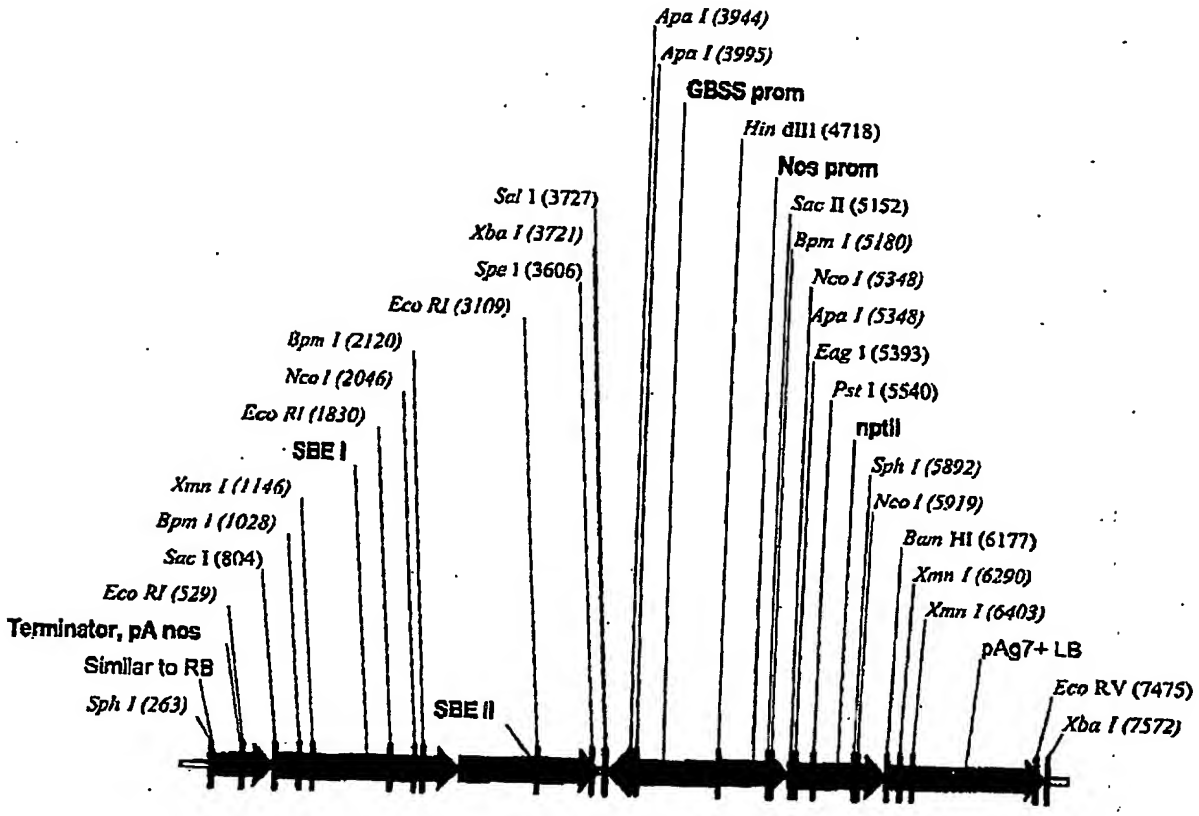

EcoRV (12286)
XmnI (11224)
pAg7
XmnI (11111)
BamHI (10998)
SphI (10713)
nptII
MscI (10390)
PstI (10361)
ApaI (10169)
SacII (9973)
Pnos
HindIII (9538)
GBSS prom
ApaI (8767)
SalI (8547)
XbaI (8541)
BamHI (8535)
SmaI (8532)
KpnI (8530)
SacI (8524)
Nosterminator
EcoRI (8249)
SphI (7983)
Misc Feature 15
ColE1 ori
Misc Feature 16
oriV
MscI (5372)
NotI (5515)
SacII (5521)
EcoRV (4444)
Misc Feature 2
PstI (3707)
MscI (3760)
pGPTV-kan backbone
EcoRV (2643)
Misc Feature 4
Misc Feature 5
pHo3.1
12379 bp

Figure 9



pHAbe12A

7756 bp

Enhanced amylose production in plants

Abstract

The invention relates to methods for increasing the amylose content in plants, preferably in potato plants, by expressing a starch biosynthesis enhancing protein. The invention furthermore relates to an expression cassette expressing the polypeptide in potato plants, preferably in the tubers, the transgenic plants expressing the polypeptide and to the use of said transgenic plants for the production of fine chemicals especially other than native starches.

PCT/EP2004/002096

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